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LUMBAR PUNCTURE

Its Anatomical and Physiological Relations.
Technique. Methods of Investigation. Diagnostic
Value and Therapeutical Applications

WITH AN APPENDIX ON ENCEPHALOGRAPHY AND PUNCTURE OF
THE CISTERNA

BY

MARTIN PAPPENHEIM, M.D.

*Professor at the University of Vienna ;
Medical Superintendent of the Neurological Department,
Municipal Infirmary, Vienna*

TRANSLATED BY GEORGE CAFFREY

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PREFACE.

FIFTEEN years' interest in the subject is not the only foundation of the present book. It is also based on the experience gained during a special teaching activity of several years. In the course of this activity I was able to observe that among the many students and practitioners attending lectures and post-graduate courses in the methods of investigating the cerebro-spinal fluid, one or another who had already studied the subject for some time, and who had employed his findings in scientific research, proved guilty of making gross errors of technique. Co-workers with extensive experience, and names honoured in the profession, would carry out some of the tests in a manner that excluded all understanding of essentials in the reactions. Thus I learnt why the literature of the subject sometimes contains results which contradict all other experience—results that are, therefore, manifestly erroneous.

Continual contact with students, however, has taught me the importance of giving due consideration to trifles, and of giving special prominence during the instruction to certain details of technique, which has given to my book, I think, in some respects a personal touch. The description of the albumin tests, for instance, may appear too detailed to some. But I know that beginners are only able to acquire the technique by thinking out things for themselves, not by offering them tables and charts like those of Grahe, which wander from one textbook to another, misprints as well. A foremost place has been given to the quite definite routine performance of the tests, for I consider that for practical diagnostic purposes, for which I have been in the main concerned, the

selection of individually adapted tests urged by some writers is usually unnecessary. I am convinced that an adequate picture of the pathological alterations of the spinal fluid will generally be obtained by following the routine here described.

Proceeding on these lines I have only dealt with the more important methods of investigation, while including some, such as the Wassermann reaction and the hæmolysin test, which the practitioner does not carry out himself, but which I also regularly demonstrate; for I consider that the practitioner should understand these reactions sufficiently to enable him to interpret the results of institutional investigations, subject, of course, to his being advised of the special conditions of investigation there obtaining.

A number of interesting modifications have also been referred to, as they serve to illustrate the objects aimed at in the reactions and to facilitate a comprehension of the methods employed. I have also thought it necessary not to neglect the anatomy and physiology of the cerebro-spinal fluid and its spaces, likewise the theoretical significance of the various modes of investigation, although, in accordance with the state of our knowledge, some unverified results have necessarily been included.

With the rapid spread of lumbar puncture some aspects of our knowledge of the cerebro-spinal fluid are continually progressing, and comprehensive descriptions therefore quickly become out of date. Responding to the wish, however, of a large number of colleagues who, notwithstanding the existence of a few quite good monographs on the spinal fluid, requested me to write the substance of my lectures, I feel justified in assuming that this book fulfils a certain need, and that it may perhaps prove of interest beyond the immediate circle attending my courses.

THE AUTHOR.

Vienna, December, 1921.

PREFACE TO THE ENGLISH EDITION.

A YEAR has elapsed since the German edition of this book was published. Numerous papers which have appeared in the interval have induced me to undertake a number of additions to the work. I have also given full consideration to the two important works of Lange and Weigeldt, without, however, engaging in controversy where Lange's view differs from mine. The appendix on encephalography and puncture of the cisterna has been newly added.

THE AUTHOR.

Vienna, December, 1923.



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LUMBAR PUNCTURE.

CHAPTER I.

THE CEREBRO-SPINAL FLUID. ANATOMICAL AND PHYSIOLOGICAL RELATIONS. ITS ORIGIN, ABSORPTION AND MOVEMENT. PERMEABILITY.

THE term lumbar puncture denotes the withdrawal of cerebro-spinal fluid¹ by the introduction of a cannula in the region of the middle or the lower lumbar vertebræ. This simple and comparatively safe method was devised by Quincke, who published its details in 1891. The method has rendered it possible to obtain larger amounts of cerebro-spinal fluid for investigation purposes, and it has enriched the medical sciences to a very considerable degree.

Lumbar puncture was originally employed mainly for therapeutic purposes, and for differentiating between the various forms of meningitis by the bacteriological examination of the fluid. Quincke had already stated in his earliest publication, however, that he "would not hesitate to employ lumbar puncture for diagnostic purposes in certain cases." At the beginning of the present century a greater diagnostic value, especially in syphilitic affections of the central nervous system, accrued to investigations of the cerebro-spinal fluid, owing to French workers, who had turned their attention to the cells and the albumin content. During the past twenty

¹ This name was first given by Magendie (1825); the subarachnoid fluid was first described by Valsalva (1666-1723).

years the sphere of usefulness of lumbar puncture has been extended both for diagnosis and for treatment. Spinal anaesthesia, moreover, represents a special form of its application.

The technique of lumbar puncture will be better understood after a brief consideration of certain anatomical points. The spinal cord is ensheathed by three membranes. The innermost is the very vascular pia, which is a soft membrane rich in blood-vessels and is closely attached to the spinal cord. Next to this is the delicate transparent arachnoid which encloses the spinal cord loosely in the form of a sac. A considerable space

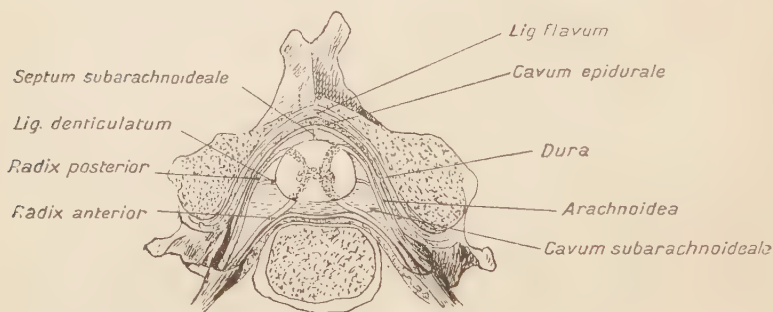


FIG. 1.

thus exists between the pia and the arachnoid—the subarachnoid space—which is traversed by fine strands of connective tissue, dividing the space into numerous intercommunicating compartments. This space contains subarachnoid, i.e., cerebro-spinal, fluid. The outermost membrane is the very fibrous dura mater, which has a shiny, tendinous appearance, and constitutes the firm sheath of the spinal cord. The internal layer of this structure is the dura mater proper, and between this and the arachnoid is an interstitial space—the subdural space—which, however, is of no practical importance, so that the arachnoid and dura may be regarded as a single sheath.

The exterior or periosteal layer of the dura—the endorhachis—which is attached to the bone, forms with the internal layer the epidural space. This space contains loose connective-tissue, fatty-tissue, large venous plexuses and an extensive lacunar arrangement of epidural lymph-spaces. In the skull the conditions are quite different, for the dura is not divisible into an internal and external layer; it is a single membrane, closely attached to the margin of the foramen magnum and quite adherent to the bony skull.



FIG. 2.

Of great significance is the fact that the sac formed by the arachnoid and the dura at its lower end, forming the cisterna terminalis of Dönitz, extends considerably beyond the lower termination of the spinal cord. It dwindles to a conical point at the level of the second or third sacral vertebra, while the spinal cord does not extend beyond the conus terminalis at the level of the second or third lumbar vertebra.

The spinal cord is suspended in the dural sac, floating, as it were, in a vessel filled with liquid. There are two

suspensory bands, first the ligamentum denticulatum, which is a single layer beginning at the pia and attached to the arachnoid by 19 to 23 points, thus imperfectly separating the lumbar space into an anterior and a posterior portion. The other band is the subarachnoid septum, which extends along the dorsal fissure of the cord, joining the pia with the arachnoid and more or less completely dividing the dorsal portion of the subarachnoid space into a right and a left half.

The subarachnoid spaces of the brain and the cord are in free communication with each other; likewise the ventricles of the brain with the subarachnoid space. The latter communications occur at three places, viz., (1) through the unpaired Magendie's foramen, which is situated at the posterior end of the fourth ventricle directly above the calamus scriptorius; and (2) at the two lateral apertures of Luschka, which open at the lateral convexities of the fourth ventricle at the flocculus.

The normal volume of the cerebro-spinal fluid is about 150 c.c., more than half of which falls to the share of the spinal subarachnoid space, and about a quarter to the ventricles. Sometimes in old age and in pathological conditions the amount of fluid is very considerably increased.¹ Excepting the cisternæ² the layer of fluid

¹ In order to determine the volume of the fluid it has been suggested to inject 3 c.c. of a 2 per cent. solution of indigo-carminé intraspinally and then to estimate the coloration of the fluid (Baruch).

Weigeldt investigated seven normal cases post mortem and found the average volume of the cerebro-spinal fluid to be 146 c.c. (118-194 c.c.); the ventricles contained 36 c.c. (27-43 c.c.); the cisterna terminalis 19 c.c. (17-23 c.c.); the whole of the spinal sac 77 (68-89 c.c.), i.e., about 3 c.c. for each vertebra. The cortical and basal subarachnoid spaces contained 33 c.c. Bingel was able by insufflation of air to measure the exact volume below a totally occlusive lesion and thus to locate its situation (*D. Ztschr. f. Nhk.*, 72, p. 359).

² These are the larger spaces situated dorsally between the medulla oblongata and the cerebellum, ventrally from the medulla to the chiasma, also in the Sylvian fossa and above the corpus callosum.

around the brain is only interstitial in amount. In the region of the cord it is variable and in some places it attains a depth of a few millimetres. The main functions of the cerebro-spinal fluid appear to be to protect the central nervous system against mechanical shocks and to maintain the osmotic equilibrium of the nerve cells. It may also assist in removing cleavage products of the central nervous system. Some writers consider that the fluid plays a part in the nutrition of the brain and cord and in biological processes; these functions, however, are still questionable, especially the former, in view of the small amount of nutrient substances the fluid contains.

The fluid in all probability arises from the vascular system, and from the part that originates from the choroid artery. It constitutes the choroid plexus¹ of the cerebral ventricles, and owing to its very villous character it possesses a considerable superficial area—said to be about one square metre.

The fluid which thus originates is probably reinforced by lymph from the brain and spinal cord, which is said to pour from minute lymph-stomata directly into the subarachnoid space. Some writers ascribe some importance to the endothelium of the subarachnoid space as a source of the fluid; others limit this occurrence to pathological conditions, while excluding it from the normal.

Opinions are still somewhat divided on the question as to the manner in which the fluid originates from the plexus. The view that it is a "simple transudation" seems to have few adherents. It does not partake of the character of a transudation, for substances such as immune bodies, which would pass over in a transudate, do not appear at all in the normal fluid, or only in the

¹ The plexuses are well supplied with nerves of sympathetic and parasympathetic origin, being derived partly from the carotid and vertebral plexuses and partly from delicate branches of the third, sixth, ninth, tenth, eleventh and twelfth cranial nerves (Stöhr).

minutest quantities. As opposed to transudates, moreover, the chemical composition of the fluid, both in respect of its albumin-content and its inorganic salts, is different from that of blood-serum. Transudation may, however, play an important part in certain pathological states, e.g., when a part of the subarachnoid space is barred by a space-reducing lesion, or when there is congestion in the region of the superior vena cava. The view now generally accepted is that the cerebro-spinal fluid is to be regarded as a product of secretion—a view already held by Luschka (1855). This view is supported by the work of certain investigators who found that certain substances, such as pilocarpine, theobromine, ether, epinephrin, promote the formation of the fluid, while others, such as atropine, hyoscine, thyroid extract and plexus extract, hinder its formation—although this action is not so well marked as is the case with ordinary glands. The effect of pilocarpine on the choroid plexus is to produce a swelling and hyperæmia, an increase in size of the plexus cells, and changes in their appearance. Weed and McKibben were able to influence the production of cerebro-spinal fluid in cats by the intravenous injection of hypo- and hypertonic solutions, and Clairmont claims to have succeeded in diminishing the accumulation of the fluid in a case of “meningism,” following an injury to the skull, by repeated intravenous injection of a hypertonic solution of common salt (100 c.c. of a 15 per cent. solution). Weigeldt does not consider that the experiments recorded in the literature are conclusive, for an apparent increase in the cerebro-spinal fluid could be due to a venous hyperæmia which had merely provoked an increased outflow of the already formed fluid.

The process of secretion from the choroid plexus, however, cannot be precisely the same as that obtaining in a true gland, for the cerebro-spinal fluid contains no constituents which are not already present in the blood-serum, which is not the case with true glandular secretions.

The process in the choroid plexus has therefore been imagined to take place in different ways. Thus Fleischmann speaks of a filtration process whereby a specific cell-activity in the plexus determines the absorption of all substances which could be deleterious to the central nervous system if they gained access to it ("adsorptive secretion"). Mestrezat refers to a dialysis of blood-plasma through a specific epithelium. Lange accepts this view as far as the dialysable portion of the fluid is concerned, but he holds that the non-dialysable portion of the normal fluid—the colloidal solution of albumins and the corpuscular elements—has an endogenous origin, the cells being derived from the moulting of the epithelium, and the albumin from the disintegration of these cells. He considers the importance of the plexus to lie only in the greatly increased superficial area of the dialysing membrane, in consequence of which a considerable amount of fresh cerebro-spinal fluid can be rapidly formed in case of need. However this may be, the normal function of these cerebral plexuses is probably to regulate in a definite manner the passage of certain substances from the blood into the cerebro-spinal fluid by specific cell-activity, and to prevent the passage of abnormal substances introduced into the body from without, or formed within the body, so that a fairly constant composition of the fluid is maintained. On the other hand it appears that substances from the cerebro-spinal fluid can pass without restriction into the bloodstream.¹

As long as the meningeal plexuses are intact very few abnormal constituents of the blood are found in the

¹ This point is contested by Lange, who says that this statement is based on animal experimentation—the barrier between the cerebro-spinal fluid and the blood being overcome forcibly. In ordinary circumstances, however, the passage from the fluid to the blood is governed by the same factors as those which apply in the reverse direction, i.e., those of dialysis in a membrane with constant diameter of its pores.

cerebro-spinal fluid, and then only in comparatively small amounts after considerable accumulation in the blood. Such drugs as chloroform, bromine (after prolonged use, when it can replace a normal constituent of the body fluids, viz., chlorine), urotropin (or its decomposition product formaldehyde), can pass into the cerebro-spinal fluid in appreciable quantities, while other drugs do not pass over, or only in traces. Among pathological constituents of the blood-stream which can find their way into the subarachnoid space are bile-pigment (but only after prolonged jaundice), diacetic acid, acetone, and lactic acid. Nor are micro-organisms as a rule allowed to pass through the healthy meninges¹; and immune bodies which circulate in the blood have only been found in the normal fluid in very high dilution (e.g., typhoid agglutinins in the proportion of 1 : 800 as compared with the amount present in the blood).² Even very diffusible substances, e.g., uranin, only enter the spinal fluid in small amount and after a considerable time. Lange asserts that the laws which in general apply to the passage of dialysable substances through membranes hold good for the passage of substances through the meninges; thus volatile substances, such as chloroform, acetone and alcohol, pass most readily; for the diffusion of salts the law which relates to the readily dissolving series applies (e.g., urea, sulphocyanates, iodides, nitrates, chlorides, sulphates). The state of permeability can be tested by Mestrezat's method: 1 gram. of sodium nitrate per kilogramme of body-weight is given by the mouth three hours before performing a puncture, and the amount

¹ But micro-organisms such as the bacilli of typhoid and of diphtheria, and *Spirochæta pallida*, are occasionally found in the fluid, even with apparently intact meninges; the specific organism of relapsing fever, after its injection for therapeutic purposes, moreover, can invade the cerebro-spinal fluid when the meninges are diseased, as in general paralysis (Plaut and Steiner).

² It is quite likely, however, that there was an abnormal permeability in all these cases, as is usual in typhoid.

present in the fluid is estimated colorimetrically with diphenylamin. The amount found in normal fluid, with or without previous administration of the salt, is 7-10 mg. per cent. ; in meningitis, however, from 70-80 mg. per cent.

In pathological conditions of the meninges there is a stronger tendency for substances circulating in the blood to pass into the spinal fluid. This is probably due both to damage done to the plexus cells and to the abnormal permeability of the meninges resulting from the inflammatory conditions. But even in these conditions the function of the dividing wall is not entirely abolished, for the passage out of the blood-stream never becomes uniform for all substances, and even with severe damage to the meninges the permeability is comparatively low, e.g., immune bodies in no higher dilution than a proportion of 1:100 as compared with their amount in the blood.

The absorption of the cerebro-spinal fluid takes place partly through the extensive venous system of the cranio-spinal cavity, and partly through the sheaths of the nerve-roots and trunks which issue from the central nervous system. In the skull, the arachnoid villi which open into the sinus participate in the process and the absorption is mainly into the veins ; in the spinal cavity the absorption is chiefly into the nerve sheaths. There is also some absorption through the perivascular spaces, by which the fluid reaches the lymphatic vessels of the meninges and the lymph-stream ; in Monakow's view this path serves for the removal of insoluble substances, which is effected by special cells. There is no consensus of opinion as to whether the bulk of the cerebro-spinal fluid filters away in the region of the brain or in that of the spinal cord.

The equilibrium between the production and the absorption of the fluid appears to be finely adjusted in normal conditions. In pathological conditions this

equilibrium is subject to disturbances which can give rise to an increase in the volume and in the pressure. The amount of fluid may be increased through over-secretion in the plexus, through excessive permeability of the meningeal vessel-walls, perhaps through hyper-secretion of the endothelium and an increased accession of lymph; or by diminished outflow due to obstruction of the efferent lymph-vessels through fibrous growth of connective tissue or cellular infiltration in the loose arachnoid tissue, or to lessened absorption through the blood-vessels owing to compression of the veins. On the other hand, certain after-effects of lumbar puncture may in some cases be due to an insufficient production of cerebro-spinal fluid.

So far as the, as yet incomplete, researches permit an opinion to be formed, this fluid, interposed in the blood-stream between the choroid artery and plexus on one hand, and the veins of the central organ on the other, although apparently possessing no real circulation, has a certain undulatory movement, which is imparted to it by the pulsation of the cerebral arteries and the alternating state of distension of the cerebral veins—which collapse with inspiration and distend on expiration. This movement is also aided by the increase of volume of the brain during systole, and its decrease during diastole. As a result the fluid leaves the ventricles by way of the passages leading out of the fourth ventricle into the cerebellar cistern. Thence a portion of the fluid passes into the cisterna chiasmatica and the cerebral subarachnoid space, while another portion flows into the subarachnoid space of the spinal cord, room being made for the fluid by the emptying of the venous plexus which surrounds the cord. With the return undulation the fluid does not pass as far as the ventricle—this is substantiated, e.g., by the occurrence of clear fluid in cases of subarachnoid hæmorrhage (Bungart); but having reached the base of the skull it flows into the subarachnoid space of this region

where it meets with much less resistance than in the narrow channels which form the apertures leading to the ventricle.¹

The movement of the cerebro-spinal fluid is probably very slow. Mestrezat's statement to the effect that the fluid is subject to renewal six or seven times during the twenty-four hours is manifestly incorrect for normal conditions. Gennerich, relying on his observations in intra-spinal salvarsan treatment (soda-salvarsan producing turbidity in the spinal fluid), states that in normal conditions about a fortnight is required for the entire renewal of the fluid. Weigeldt's investigations by means of encephalography in normal cases showed that after the withdrawal of 100 c.c. of cerebro-spinal fluid no air was demonstrable by X-rays after an average of fifty-five hours; thus 100 c.c. of fluid was renewed in two to three days; Weigeldt conjectures, however, that without previous insufflation of air the renewal of spinal fluid takes place at a still slower rate. In pathological conditions, when large amounts of fluid are withdrawn by lumbar puncture, or especially if a fistula persists at the site of the puncture, the renewal of the fluid is much more rapid. The fluid also seems to form more quickly when there is a congestion of the head. Weigeldt found by experiment, and also in cases of congestion of the superior vena cava owing to mediastinal tumours, that the spinal fluid had an abnormally low specific gravity and an abnormally low albuminous content.

It will be readily understood from the foregoing description of the movements of the spinal fluid that taking the spinal fluid space as a whole, no perfect

¹ The gases employed in encephalography pass through these openings, however. Eskuchen assumes the existence of a "circulation" of the fluid from below upwards, as he has recently been able to show by puncture of the cerebellar cistern (cf. Appendix) that serum, which is heavier than spinal fluid, travelled up the spinal subarachnoid space from the lumbar region to the occipital foramen within twenty-four minutes.

mixture of the fluid takes place, for which reason the composition of the fluid is not alike throughout. Fischer, who long ago first pointed this out, asserted that an examination of the fluid could only yield evidence of pathological changes at the site of the puncture—which goes a little too far. As Weigeldt describes, there are other circumstances besides the position of a lesion which play a part: (1) The sedimentation of the fluid, which finds expression in a greater number of cells being contained in the first portion of the fluid than in the later portions; (2) local differences in respect of the accession of lymph on one hand, and the absorption of fluid on the other, which, in view of the difference of composition of these two liquids, can give rise to chemical differences in the composition of the spinal fluid; (3) the pressure-conditions within the subarachnoid space—for when the space is tense the uniform mixture of the fluid would be less complete, and with lower pressure it would be more thorough; (4) the extent of bodily movements which would aid this; and other factors.

The differences in composition of the various portions of the cerebro-spinal fluid are, however, not as a rule considerable, and they are certainly not decisive in questions of diagnosis; but occasionally there are great differences in the cell-content, and also—to judge from the interesting evidence which Weigeldt has been able to produce as a result of difficult investigations by puncturing the cerebro-spinal space in various regions—there are differences in the specific gravity, in the albumin content, the gold reaction, the Wassermann reaction, and even in the tubercle bacilli content.¹ Weigeldt has also shown

¹ In one case of tubercular meningitis, Weigeldt found 389 cells [per cubic millimetre] and 1.33 per thousand albumin in the first portion of cerebro-spinal fluid; but after the withdrawal of 60 c.c. of the fluid there were 1,004 cells and the albumin was 1.66 per thousand. In another case of the same disease there was an absence of bacilli in the first portion, and also after the withdrawal of 25 c.c.; but they were found after the withdrawal of 50 c.c. In another case of tuber-

that the ventricular fluid is greatly different in composition from that of the spinal cavity. In the former, the albuminous substances vary from 0·125 to 0·166 per thousand; in the latter the albumin varies from 0·166 per thousand to 0·333 per thousand. The specific gravity varies from 1002·1 in the ventricles to 1007·3 in the lumbar fluid.

cular meningitis, puncture in different situations gave the following results: ventricular fluid, no cells; albumin 0·42 per thousand; in the fluid of the cisterna 2,940 cells, very numerous bacilli, albumin 3·5 per thousand; in the lumbar fluid 927 cells, some bacilli, albumin 2·25 per thousand. In a case of impacted gunshot wound of the skull the findings were as follows: ventricular fluid 4 cells, albumin 1·16 per thousand; in the fluid of the cisterna 257 cells, 2·25 per thousand albumin; in the lumbar fluid 172 cells and 1·66 per thousand albumin. In a case of septicæmia, with numerous cerebral abscesses, the ventricular fluid contained 2,867 cells, and the albumin content was 1·66 per thousand; in the cisterna there were 381 cells and the albumin 1 per thousand; in the lumbar fluid 63 cells and 1 per thousand albumin.

CHAPTER II.

THE TECHNIQUE OF LUMBAR PUNCTURE.

THE advantage of Quincke's method of lumbar puncture consists in the fact that the puncture through which the cerebro-spinal fluid is withdrawn is made in a region below the termination of the spinal cord, i.e., below the second lumbar vertebra, where the nerve roots of the lower spinal cord segments form the cauda equina and float in the fluid. Only the insignificant filum terminale of the cord still continues in this region. No injury can befall the cord in this situation; the nerve roots usually escape the needle, and if they should happen to be wounded, the resulting slight disturbances are at the utmost of a quite transient character. But it is quite practicable to perform the puncture in other situations in the spinal subarachnoid space. Weigeldt recommends this procedure to be adopted in cases wherein the establishment of a diagnosis requires fluid to be taken from different situations. In these circumstances it is necessary that the patient should be completely at rest, and the needle must be carefully directed, and precautiously guided by the sense of touch. If the cervical spine is to be punctured, the approach must be lateral; in the thoracic region the puncture usually presents no great difficulties if the back is bent to an extreme degree of kyphosis, and the needle is introduced in the direction of the head at an acute angle to the surface of the back.

The puncture in the lumbar region is made in the space between the third and fourth lumbar spinous processes. This space is in the line joining the posterior superior iliac spines, for this line passes just above the

fourth lumbar spinous process, and therefore the required space is just above this. The puncture may be made in an intervertebral space a vertebra higher or lower, even between the fifth lumbar vertebra and the sacrum, although the procedure is more difficult. In certain circumstances a lower puncture is advantageous, because if the cerebro-spinal fluid obtained is artificially mixed with blood, a rapid puncture can be made higher up and



FIG. 3.

fluid obtained which is free from blood. But on the whole, punctures other than in the usual situation are not to be recommended, because of the greater difficulty with which they are attended.

The patient must be completely undressed for the puncture, and should be lying preferably on the right side. If the patient is lying on the left side the operator must stand with his face towards the patient's feet. The puncture should only be made in the sitting posture in exceptional cases, e.g., when it has failed in the lateral

position, or when the patient is restless but can be kept still in the sitting posture without using very much force. The lateral position is more comfortable for the patient, and it also yields more accurate pressure-estimations, except in certain cases. The patient's back must be bent up as completely as possible, in order that the spinous processes should separate widely from one another, and this is facilitated by the patient fully bending his hips and head. The patient may support his legs with his folded arms, and care must be taken that the shoulder on which the patient lies does not project backwards. A nurse should be available to maintain the patient in this position, giving as much assistance as may be required for the purpose. Quiet and reasonable patients require no assistance, but it is desirable to warn them just before the actual puncture not to draw in the back when the needle is felt. The patient's head must be at the same level as the body, and therefore must not lie on a cushion. In cases where special care is demanded the head must lie lower than the body. If the patient is in a sitting posture, the upper part of the trunk must be bent sharply forward and should be supported by a nurse standing in front of the patient with her arms passed between his axillæ. Or the patient can bend forwards with his arms between his legs, grasping the legs of the chair, and thus support himself. The most convenient seat is a stool (i.e., without a back); or a chair with the back placed to the side may be used.

The needles usually employed for puncture are made of steel and nickel-plated, the length of the needle itself being 9 to 12 cm., and its lumen 1 to $1\frac{1}{2}$ mm. The point must be comparatively short, smoothly and straightly ground and extremely sharp. After being used the point should be carefully resharpened on an oilstone. Wider needles are apt to injure the blood-vessels, and to contaminate the cerebro-spinal fluid with blood. They are also more liable to cause unpleasant sequelæ. The

needle is provided with a trocar or stylet which possesses a handle, thus allowing of a firm grip, and a stop that fits in a groove at the posterior end of the needle. Schottmüller recommends a needle with a very large basal attachment, when the cerebro-spinal fluid is required for bacteriological examination, so that it can be grasped conveniently without touching the aperture of the needle and so compromising the asepticity of the fluid. For the purpose of estimating the pressure of the cerebro-spinal fluid, the needle depicted in fig. 4 is most useful. It is provided with a branch-piece at an angle of 60° ; both the branch and the needle itself can be closed by stopcocks. Shorter and narrower needles

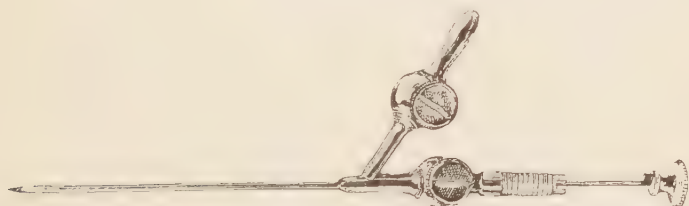


FIG. 4.

should be employed for children, and it is wise to be furnished with a variety of sizes, even for adults. The needles should be placed in tin or cardboard boxes and placed in a hot-air sterilizer; they will bear the necessary heat quite well according to my experience. Lange takes the needles to pieces, puts them in a large test-tube of alcohol, and boils them over an open flame or in a water-bath. The alcohol is then poured off and the needles are dried in a hot-air sterilizer at a moderate temperature. Care must be taken that the alcohol is completely evaporated. The needles are then placed in a test-tube which is closed with sterile cotton wool. The needles must be syringed through with water and alcohol *immediately* after use, to prevent rusting. Nickel

needles do not rust, but they have the disadvantage that the cerebro-spinal fluid does not flow freely through, owing to the thickness of their walls. Platinum-iridium needles are very thin and are therefore less painful for patients, but they are not in vogue owing to their cost. These needles are flexible and therefore do not break if subjected to a great strain, but they can get bent out of shape, and twisted sufficiently to break, if care be not taken. If a needle should break, it is usually easy to extract it with forceps; sometimes a skin incision may be necessary before the needle can be seized. One case is recorded in which it was necessary to chisel away a spinous process.

Only rarely is it necessary to pacify very restless patients with doses of hyoscine hydrobromide ($\frac{1}{60}$ gr. subcutaneously), or by the administration of paraldehyde (1 to 2 fl. dr. in 3 fl. oz. of water, by the mouth), an hour before the puncture, or by a light ether anaesthesia. An injection of morphia ($\frac{1}{12}$ to $\frac{1}{6}$ gr.) may be given with advantage to very sensitive patients fifteen minutes before undertaking the lumbar puncture. Local anaesthesia by means of an ethyl chloride spray is unnecessary; not only does it render the operation more difficult, but in subjects hypersensitive to cold it occasions as much pain as the puncture itself. Preferable is a local anaesthesia carried out in the following manner: 2 c.c. of a $\frac{1}{2}$ per cent. solution of novocaine is drawn into the syringe, $\frac{1}{2}$ c.c. of which is injected under the skin at the point intended to puncture, and the remainder is injected through the wheal thus produced after having introduced the needle vertically to a depth of about 2 cm.

A scheme should be drawn up beforehand of the examinations to be undertaken and the quantities of fluid required. In employing the modes of procedure described in this book (viz., cell-counting in the chamber; globulin estimation by the Nonne-Apelt method, combined

with the estimation by means of submerged layers of ammonium sulphate solution, and by Pándy's method; the total estimation of albumin by the method of Brandberg-Pfaundler; the colloidal gold test; the Wassermann reaction, and perhaps the qualitative cell examination in a film preparation) the following amounts of cerebro-spinal fluid will usually prove sufficient¹: For cell-counting, 0.5 to 0.6 c.c.; for the albumin and the colloid reactions, about 2 c.c., and approximately the same volume for the Wassermann reaction. Should the last-named be carried out with active and inactivated fluid, 3 to 4 c.c. will be necessary. The usual procedure during the puncture for diagnostic purposes is as follows: The quantity of fluid to be employed for cell-counting is allowed to drop directly out of the cannula into a watch-glass, and the mixture with the counting-stain is prepared. The initial pressure is then measured. (Only in exceptional cases to be mentioned later should one commence with the pressure measurement.) The fluid within the upright tube is then allowed to flow into a centrifuge tube (fig. 7). Then 2 c.c. of fluid is withdrawn into a small tube for the albumin investigations and the gold test—this is collected directly from the cannula to avoid its passing through the connecting-tube used in pressure measurement. Fluid is now added to that contained in the centrifuge tube to the amount necessary for the Wassermann reaction, subtracting, however, the probable amount that will remain in the upright tube after having measured the end-pressure. The end-pressure is now measured; and if a second cell-count of a later portion of fluid is desired, a few more drops of fluid may now be collected in a watch-glass and

¹ If only very minute quantities of fluid are available Plaut's micro-method of investigating the cerebro-spinal and ventricular fluids may be used. In this method micro-pipettes are used, and the drop method is employed, and the reactions are performed in capillary tubes with minimal amounts. (*Zeitschr. f. d. ges. Neur. u. Psych.*, 1921, vol. 65.)

mixed with the staining solution. The fluid remaining in the upright limb is now added to that in the centrifuge tube, and after having closed both the cocks the needle is withdrawn. Centrifugation is performed as soon as possible, pouring into a tube the supernatant fluid to be employed for the Wassermann reaction, while the sediment is utilized for studying the cells. All collecting vessels must be sterile and dry; the small tubes may be provided with a mark corresponding to the amount of fluid to be collected in them. Collecting the fluid in separate vessels has the further advantage that in case a drop of blood should enter a vessel, only this fluid would be rendered valueless, not the whole of the fluid withdrawn. When, for therapeutical reasons, larger quantities of fluid are to be removed, the employment of tubes graduated in cubic centimetres is advisable, so as to enable the fall of pressure to be kept under observation in relation to the amount of spinal fluid removed. A watch-glass for the staining solution, sterile glass ascent tubes and rubber connecting-tubes for pressure measurement, and a measuring-tape or rule, are to be put in readiness, in addition to the vessels for collecting the spinal fluid. When not in use, the puncture-needles should be kept in a dry and sterile condition; and in view of the gold test they should not be boiled with soda. For sending specimens of fluid away, Lange recommends small glass-stoppered bottles, sterilized in hot air, holding 10 c.c.; these enable an estimate to be made of the amount of fluid to be collected. The stopper is sealed with adhesive plaster, with the patient's name written on it.

When all is in readiness, an extensive area of the patient's back is cleansed with petrol or ether, shaving the surface beforehand if necessary, and the neighbourhood of the puncture-spot is painted with iodine tincture, from a hand's breadth above the crest of the ilium to a little below it, and extending laterally to the breadth of

about three spread fingers. It is also expedient to mark the upper, i.e., with the patient on the right side, the left, spina iliaca posterior superior with a streak of iodine (see fig. 3). The hands are then scrupulously cleansed—during which time the iodine tincture works its disinfection of the patient's skin—and a cannula is chosen, holding the instrument only by the attachment, not by the part that will be introduced in the body, nor at the opening where spinal fluid will flow out. In using my pattern of needle the two stopcocks should be tested prior to the puncture, and care must be taken that the cock belonging to the side branch is closed when the cannula is introduced. Some authors claim that greater sterility and less displacement of the needle by folds of tissue are obtained when an incision is made before puncturing. The method is not as a rule necessary, but it may be employed with advantage for intraspinal salvarsan-treatment. Gennerich cleanses the surface with soap, followed by alcohol and ether. He then marks a cross with narrow streaks of iodine to indicate the point of intersection where the needle is to be introduced, and after freezing with ethyl chloride, or raising a superficial wheal by injecting a local anæsthetic, he performs the incision at the point of intersection.

The spinous processes are now examined with the left forefinger, which should be iodized, and this finger is pressed into the interval between two processes, generally those of the third and fourth lumbar vertebræ. The needle is then introduced close up to the forefinger. The pressure of the forefinger is intended to obviate a displacement of the skin. Schönfeld intentionally displaces the skin in order that the perforations of skin and dural sac shall not be superposed, i.e., that the latter shall not open directly outwards; but this renders the operation less reliable. After long practice it is usually possible to perform the puncture without palpation with the finger, and to avoid touching the body at all with the

hand, the asepsis being thus more complete, which is of value, especially in intraspinal treatment. With corpulent individuals it is not always possible to locate the spinous processes. In such cases one may try to locate the higher and more prominent processes, gradually coming down to the lower ones. And cases occur in which the most experienced operator cannot locate the site of puncture, and is forced to puncture with uncertainty ("aufs Geratewohl").

My own procedure, which agrees with that of most writers on the subject, is whenever possible to puncture exactly in the median line. This lessens the risk of injury to the fibres of the cauda, and bleeding is less liable to occur. Only when the suspensory-system of the cord offers exceptional resistance do I perform the puncture according to Quincke's original method, viz., a little to the side of the median line, while directing the needle somewhat obliquely inwards. In the case of children, Quincke himself advised puncturing in the median line. The cannula should be held like a pen, and inserted at a right angle to the back. During its advance it is sometimes necessary to elevate a little the point of the needle in the direction of the head. When performing the puncture in the sitting posture it is necessary, owing to the outward and downward direction of the spinous processes, to direct the needle somewhat obliquely upwards with a caudalward angle of 80° to 70° ; this direction of the needle is favoured by some writers also when the patient is in the lateral position.

The layers of tissues to be perforated are as follows: The skin, the subcutaneous connective tissue, the supraspinous ligament that connects the apices of all the vertebral spinous processes; the interspinous ligament which connects the spinous process of each vertebra with that of the next, and the ligamentum flavum, which is spread between the arches of the vertebræ. All these bands constitute a more or less continuous mass

of tissue. The last layer is made up of the dura and arachnoid, which, in keeping with their tenser structure, frequently offer a marked resistance, not unlike that felt in puncturing a card. The total depth of the puncture averages in adults from 5 to 7 cm., and in children 2 cm. Bone may be occasionally encountered, in which event the needle must be slightly withdrawn and its direction changed; and at times it is necessary to remove the needle entirely and puncture anew, after a further careful digital examination, employing a fresh needle for each occasion. Very little pain is excited by a lumbar puncture when successfully performed; but boring into the periosteum can be exceedingly painful. In rare cases it is possible, after attempts in the lying position have failed, to effect a puncture in the sitting posture; and at times, e.g., in cases of great spinal curvature, one is obliged to insert the cannula at an atypical spot—either considerably to the side, or higher up. Cases are very rarely met with in which the lumbar puncture proves impossible of performance by a practised hand, or where having failed in spite of all endeavours the puncture can be effected on a later occasion in the same case. With sufficient practice the operator will be able in the great majority of cases to go clean through into the lumbar space at the first attempt—which is not only an advantage to the patient owing to there being less pain from the puncture, and because any ill effects are then apparently less frequent, but is also of importance for the investigation of the spinal fluid, for admixtures of blood more often occur the more one is obliged to alter the direction of the point in the deep-seated tissues. Sufficient practice, however, can only be gained by performing a large number of operations; and it is not infrequent to find the beginner believing he has already mastered the technique on the strength of a comparatively large number of successful punctures performed, when afterwards a larger series of failures sets in.

When it is believed that the cannula has entered the lumbar space, the stylet should not be completely drawn out, but only far enough to allow of the escape of spinal fluid. The foregoing remark applies to the use of a cannula unprovided with a stopcock; but if a stopcock is present the stylet may be completely withdrawn, and the stopcock closed immediately, if possible as soon as a single drop of spinal fluid has made its appearance. Should spinal fluid fail to flow out, the stylet may be replaced in the cannula, as this may be due—though seldom—to the presence of an obstruction in the lumen of the needle. This can sometimes be overcome by turning the cannula about its axis, or very slightly withdrawing it, after which a flow of spinal fluid can sometimes be obtained. If, however, this does not lead to the desired result, the needle must be withdrawn again entirely. If the first drops of spinal fluid contain a little admixture of blood, which may on rare occasions result from slight injury to small vessels, even when the puncture has succeeded at the first attempt, it is sometimes possible to obtain fluid free from blood by slightly revolving the instrument. At times the bleeding soon stops of itself; the reverse may also occur where the first drops of fluid are quite clear, and the later portions are transiently, or permanently, mixed with blood. If the bleeding continues, a further puncture may be performed higher up at once, perhaps changing the patient to a sitting posture. If this also shows a considerable admixture of blood with the fluid, the operation should be discontinued, and an examination made as to whether the admixture of blood is artificial or pathological; the former is generally the case. In cases of considerable artificial admixture of blood there is no alternative but to repeat the puncture a few days later. With only a slight artificial admixture of blood it still remains possible up to a certain degree to obtain useful results from the examination.

The fluid should always be allowed to flow out of the

cannula as slowly as possible, drop by drop. The rate of flow can be regulated by turning the stopcock, or in the absence of a stopcock, by adjusting the stylet. At the end of the operation the site of puncture should be painted with iodine and covered with gauze. The latter is fastened to the body with adhesive plaster or collodion.

CHAPTER III.

THE DANGERS OF LUMBAR PUNCTURE.

ALTHOUGH in the great majority of cases lumbar puncture is to be regarded as a totally harmless operation, it is necessary, when determining the indications as well as when performing the puncture, to take into account certain dangers; especially as a not inconsiderable number of fatal occurrences following upon lumbar puncture have been observed. In 1915, Schönbeck collected seventy-one such cases mentioned in the literature of the subject. In some of the cases it is probable that with a greater exercise of care, by not discharging large amounts of fluid without control, the fatal course would not have occurred. Others, moreover, will have been cases not of *propter hoc*, but of *post hoc*. Particular cases recorded in the literature of the subject seem to support this view. Thus Reichmann reports a case in which the patient was appointed for lumbar puncture, but died before the operation could be performed. Eskuchen records another case of fatal termination a few hours after an unsuccessful attempt to perform lumbar puncture, with a single insertion of the needle and not a drop of spinal fluid withdrawn. These fatal cases would undoubtedly have been attributed to lumbar puncture—had the operation been performed or been successful. The cases in which with all precautions a fatal consequence could be held in causal relationship with a lumbar puncture are decidedly rare, and they are confined without exception to cases of severe illness; for which reason these unfortunate occurrences do not justify a high estimation of the dangers attaching to

lumbar puncture, though they necessitate caution under certain pathological conditions.

Among the conditions in which lumbar puncture is certainly attended with danger is the presence of tumours of the brain in the region of the posterior cranial fossa. These sometimes bring about a cone-like compression of the medulla into the foramen magnum, and a sudden reduction of pressure by lumbar puncture can give rise to considerable compression of these regions, resulting in damage to the medulla and paralysis of the vitally important centres, especially that of respiration.¹ Internal hydrocephalus and general oedema of the brain can occasion similar exceptional conditions. In a case reported by Beck of mobile cysticercus in the fourth ventricle, lumbar puncture ended fatally by the medulla being drawn into the foramen magnum.

In other respects, danger attaches to lumbar puncture when different lesions are present, such as highly vascular tumours, recent apoplexy, the fragile blood-vessels in uræmia;² and hæmorrhages dangerous to life can super-

¹ Of interest in this connection is an observation made by Schloffer in a case of tumour of the cerebellum, in which the autopsy showed a bulbar constriction or closure ("Stöpselverschluss"), which in life manifested itself in the horizontal, dorsal position by a preponderance of ventricular pressure over that of the spinal fluid; while with the pelvis raised and the head lowered, the pressure was equalized by the brain falling backward.

Sauerbruch (*Grenzgeb.*, 1907, III suppl. vol.) was able to demonstrate by animal experiment that when the pressure in the skull was increased, a few c.c. of spinal fluid spurted out of the cannula of the lumbar puncture the moment the compression began, then not a drop further escaped until the pressure was again reduced. The compression acted on the brain equally from all sides owing to the enveloping fluid. When fluid was released below the medulla was forced into the foramen and no more fluid was able to escape.

² Of significance, perhaps, is the observation made by Kahler (*Wiener klin. Woch.*, 1920, p. 1006) that an increase of blood-pressure occurs after lumbar puncture in cases of cerebral disease accompanied by an increase of intracranial pressure. He attributes this either to stimulus of the vasomotor centre from the pressure of the medulla into the foramen magnum, or to increased irritability of this centre from functional impairment of the cortex due to pressure exerted by the brain, according to Meynert's theory of the antagonism in the supply of vessels to the cortex and bulk of the brain.

vene owing to the pressure-reducing effect of the puncture. Ponfick was the first to call attention to certain cases, such as in epidemic meningitis, in which after a rapid reduction of pressure extensive pial hæmorrhage can occur; hæmorrhage *ex vacuo* has also been spoken of. Nonne saw a fatal outcome consequent upon a lumbar puncture in a case of myxomatous tumour in the cervical region of the cord; in all probability hæmorrhage had taken place in the highly vascular tumour, with remote action on the neighbouring medulla oblongata ("Fernwirkung auf die nahe befindliche M. oblongata"), damaging its centres.

It will thus be necessary to proceed with the utmost caution in all these groups of cases, should it be decided that the puncture ought not to be abstained from. When the necessity for puncture is not immediate, as in a case of suspected tumour of the brain, the patient should lie horizontally for a day or two prior to the puncture; and after the operation the flat position should be kept for at least forty-eight hours, and the transition to the sitting and erect postures should only take place by degrees. In such cases the removal of fluid should always take place as slowly as possible. When the puncture is for diagnostic purposes, the amount of fluid taken must be limited to a minimum, and in certain cases, such as a suspected tumour of the brain, no more fluid should be removed than is necessary for measuring the pressure, and for the examination of albumin, for which purposes 1 c.c. of spinal fluid is sufficient when a mercury manometer is employed. In a given case the amount of fluid removed can be replaced by an intraspinal injection of salt solution. In cases of lumbar puncture for therapeutic purposes—and it can sometimes be a direct saving of life in just those groups of cases in which lumbar puncture is not without danger—it is best to puncture oftener and to reduce the pressure only very little during the first punctures. Contrary to the opinion of some authors, I

consider that in meningitis, too, only a moderate reduction of pressure with each puncture is indicated. In general, one may keep to the rule laid down by Quinke, that the greatest amount of spinal fluid that should be removed is when the end-pressure is about 60 per cent. of the initial pressure; e.g., with an initial pressure of 300 mm. the puncture must be discontinued when the pressure has fallen to 180 mm. It will be expedient in some cases to discharge still less fluid than that recommended by Quinke.

Even with the very frequent employment of lumbar puncture, a fatal case will hardly ever occur if these precautionary measures are taken into consideration. But, admitting that an unfavourable outcome is in a few cases scarcely to be avoided, it is not justifiable on the ground of isolated instances to warn against the employment of lumbar puncture in such cases of disease, in view of its favourable effect in many cases. As Eskuchen rightly observes, a general rejection of lumbar puncture in cases of suspected tumour of the brain means renouncing the marked advantages attending its employment in serous meningitis. And even in tumour of the brain, moreover, favourable results can at times be observed from lumbar puncture—appreciable subjective relief, and reduction of the engorged papilla.

Among the less frequent after-effects of lumbar puncture, the occasional occurrence of transient disturbances of the cranial nerves may be mentioned. Unilateral paralysis of the abducens has been known for a considerable time, and a few cases are on record, more often referring however to lumbar anæsthesia than to lumbar puncture proper. I witnessed a similar case, not recorded. More recently, cases of bilateral paralysis of the abducens have been described, as well as oculomotor paralysis, and disturbances in the region of the auditory nerve. A proportion of these cases were syphilitic, and the writers think that through the lumbar puncture a

latent syphilitic focus was rendered manifest, or that a syphilitic focus had developed at a point of least resistance. But a further proportion of the cases were not syphilitic, and these were probably due to a localized hæmorrhage in the region of the affected nerves.

But it is not really the serious mishaps that prevent lumbar puncture from being employed to the extent its importance deserves, but the much more frequent subjective after-effects which, though harmless, are often very distressing. The ill-effects due to injury to the nerve roots during lumbar puncture—pains in the legs, which may continue some time—are of less frequency. Kahler noticed in some cases, in which the puncture was accompanied by pain in the legs, a diminution of the knee reflex, and of the sensibility of the outer surface of each leg, which continued for some time. (These symptoms may have been caused by a slight mechanical injury to the nerves, or perhaps by small filiform hæmorrhages along the nerve roots, similar to those seen post mortem by Quinke.) Local pain in the lumbar region may also occur. Some neurotic cases are subject to after-effects of a psychogenic character, and neurotic accident cases are often liable to particularly unpleasant forms of reaction to lumbar puncture. Leaving, however, the less frequent minor manifestations out of account, there is a fairly characteristic syndrome, called meningism, which in a certain number of cases becomes manifest as an after-effect of the puncture.

In such cases the patient will complain of violent headache, especially at the back of the head, giddiness and nausea, and vomiting may also occur; stiff neck and Kernig's sign are seldom present. These effects usually develop a few hours subsequent to the puncture, sometimes a few days later, or they may appear very quickly. As a rule they vanish after a few days, but in exceptional instances they may continue for weeks. They occur more often in "nervous" patients, some of whom have

probably not remained quiet after the puncture. Organic neuropaths—leaving here out of account, however, cases of distress due to increase of pressure, in which lumbar puncture can favourably influence the troublesome symptoms—particularly the so-called meta-syphilitics, react less frequently with ill-effects than patients with healthy nerves. I have noticed, on the other hand, that in early cases of syphilis, without objective signs on the part of the nervous system, ill after-effects appeared more frequently when the spinal fluid was pathological than in syphilitics with normal fluid. Repeated punctures favour the development of untoward effects, which sometimes even may make their typical appearance when the punctures have been unsuccessful. The distressing symptoms are said to occur oftener after the removal of a larger amount of fluid than when only a small quantity has been taken ; they are said to be more frequent when the lumbar puncture is carried out in the sitting posture than when it is performed with the patient lying on his side. (This was my own impression years ago, though without further testing its validity. For a long time I have nearly always performed the puncture in the lying posture, and for diagnostic purposes I have only removed quite small amounts of fluid, so that I have had no further opportunity of making comparative observations.) It is characteristic of the untoward after-effects that in the horizontal position they are usually either lessened or they disappear entirely, while reappearing at every attempt to gain the sitting or erect posture.

Meningism undoubtedly occurs with less frequency if certain precautionary rules are observed during and after the puncture. First and foremost is that the puncture should not be undertaken on an ambulant patient. The punctured patient should lie for twenty-four hours afterwards in a position of complete rest ; nor should he walk from the operating table to the bed, but should be carried to it on a stretcher. He must be aided with food and

bed-pans, so as to avoid all exertion. The head must lie flat, without a pillow; and it is an advantage to elevate the lower end of the bed. Intense intellectual occupation, mental excitement, alcohol, are to be avoided. It should, however, be mentioned that many a patient, without keeping to any regulations, gets up immediately after the puncture and goes about his usual business, sometimes performing quite exerting tasks; such patients sometimes unhesitatingly tolerate repeated punctures; others again, in the same conditions, will exhibit no ill-effects one day, but upon another occasion will be distressfully affected.

The intraspinal injection of saline solution following the puncture exerts no preventive action against the occurrence of meningism. On the other hand, some writers have observed an improvement to result from the injection of copious amounts of liquid, especially from the daily injection of a litre of physiological salt solution into a vein. Injections of pilocarpine are said to effect a transient improvement of the symptoms, while injections of atropine are said to aggravate them. Should the symptoms here described make their appearance—which may not occur until the patient begins to sit up in bed, or sometimes not before he begins to move about—then the remedies mentioned may be tried. But it is advisable to keep the patient in bed if possible until the ill-effects have disappeared entirely. Anodynes such as pyramidon (0.3 to 0.6 gm.), and ice bags at the back of the neck, are often helpful. Psychic influences frequently have a favourable effect, particularly in cases which have lasted a week or more, for as a rule these are cases of psychic fixation of the original symptoms.

The data in the literature of the subject concerning the occurrence of meningism after lumbar puncture are very varied. While some writers hold the view that the after-effects may be entirely avoided, with others the data as to their frequency vary exceedingly, and figures are to be

found varying from 10 to 30 per cent., and even to 50 per cent. of all cases punctured. It is not easy to assign a reason for these great divergencies, because it is not yet known with certainty to what the after-effects are due, in spite of many attempts to explain them. Among these attempts is that of Walter, who would refer the after-effects to the tension ("Zerrung") of the cicatrice which is formed in the dura at the site of the puncture. This explanation can be at once rejected, for it is not possible to suppose such a rapid and extensive cicatrization that the ill-effects could be referable to it. On the other hand, recent writers, e.g., Gennerich and Baruch, have regarded the remaining open of the puncture-orifice as the cause of the after-effects. They consider that in some cases the orifice does not close up until long afterwards, and that the continual oozing out of the fluid through the orifice, the "drainage through the puncture-canal," brings about the evil symptoms, which do not disappear until the orifice has become sealed up. According to this view, the symptoms are to be regarded as analogous to the headaches which regularly follow the withdrawal of large amounts of fluid. This is convincingly demonstrated in Gennerich's method of intraspinal salvarsan treatment, in which the prescribed maximum reduction of fluid leads to violent headache, which begins in different patients after the removal of different amounts of fluid, and increases in intensity with continued withdrawal, while it gradually disappears when the fluid is returned to the subarachnoid space.

It is certainly the case that the orifice made by the cannula often does not close for some little time. Bungart's opinion is that as a rule it closes up in from ten to twelve days. That the orifice does not close immediately can be seen from the circumstance that the dressings placed over the puncture spot are often still damp after some days, and that at times an œdema develops in the neighbourhood of the site of puncture

which may remain for a considerable time. In such cases, however, one does not always observe the phenomenon of meningism, and, as Bungart rightly remarks, during operations on the brain and cord, real streams of spinal fluid are shed, both during the operation and afterwards, without the symptoms under consideration making their appearance. Neither does the observation hold good which Hosemann brings forward in favour of the causal significance of drainage through the orifice, to the effect that with lumbar punctures which had been made some little time after a lumbar anæsthesia had been performed, either the fluid obtained was often under a very low pressure, or there was no fluid at all; which the author ascribes to the reduction of pressure consequent upon a continuous drainage of fluid. Bungart quite justly interprets Hosemann's findings as the result of local adhesions of the meninges in consequence of irritation from inferior anæsthetic preparations; by puncturing higher up he was unable to confirm Hosemann's observations.

It seems most probable that the drainage through the puncture canal does not as a rule lead to ill-effects, because presumably the loss of fluid is comparatively inconsiderable, and is compensated by the regulated production of fluid. This view is also supported by the fact that in cases of injury to the head with defects of the bony skull, the distended integument in the neighbourhood of the injury sinks in during the puncture, but reaches its former level again very quickly. An insufficient production of fluid after a puncture may perhaps be of importance in exceptional cases. In other cases, according to Bungart's observations, that were also supported by his post-mortem findings in a hydrocephalic child, an abnormal communication between ventricle and sub-arachnoid space, brought about by injury to the head, can lead to abnormally large issues of fluid from the puncture canal, and to a deep retraction of the scar,

which is accompanied by severe after-symptoms. On this account, Bungart advises most careful observation for a period of ten to twelve days after the puncture, in cases of injury to the head. In general, however, I do not consider the drainage of fluid from the puncture canal an adequate explanation of the after-effects.

I believe that from the foregoing evidence I am justified in saying that the ill-effects consequent upon lumbar puncture are referable to a local irritation of the meninges caused by the puncture. Zaloziecki communicated to me particulars of a personal observation, also cited by Weigeldt, to the effect that a meningism following a lumbar puncture performed upon himself disappeared after a second puncture. Rost has placed significance in this respect upon the occurrence of a local hyperæmia with perhaps slight extravasation of blood. And Quincke sought to explain some cases by local inflammatory changes, calling attention to the fact, which had already been mentioned by Nissl, and has been strongly stressed more recently by Schönfeld, that in some cases of repetition of lumbar puncture within a short period an originally quite normal fluid afterwards gives pathological cell- and protein-findings. Weigeldt's physiological investigations show that after repeated removal the fluid is rendered poorer in cells and albumin. In explanation of cases without such sharply defined pathological alterations, which need not necessarily give rise, of course, to ill-effects, it will be permissible to suppose that changes in the concentration of the fluid, not demonstrable by the customary tests, lead to osmotic disturbances in the cells, and thence to the ill-effects.

The favourable influence of secretion-promoting injections of salt solution and pilocarpine, which is put forward in support of the "drainage theory," on the view that it promotes a quicker renewal of the leaking fluid, can be made to agree with the above explanation by conceiving that the improvement in the conditions is

brought about, not by the resulting increase of fluid, but by the restoration of the fluid to its normal concentration. And the special tendency to after-effects shown in certain groups of disease, such as neuropaths, early cases of syphilis with pathological spinal fluid, must be accounted for by assuming a special irritability of the meninges in these cases, or a special susceptibility to injuries of the meninges caused by the puncture. In this connection I believe I have observed in cases of infectious lesions of the ear, in which a "réaction à distance" of the non-infected meninges often occurs spontaneously, that a repetition of the lumbar puncture shows comparatively frequent alterations in the previously normal fluid.

More recently the ill-effects observed in some quarters after lumbar anaesthesia, and apparently also after lumbar puncture, have shown a decided increase. This circumstance would also seem to support the view here advanced, as puncture needles of inferior material would presumably increase the amount of irritation of the meninges, and, on the other hand, perhaps a more widespread employment of lumbar puncture has induced a laxer observance of the essential precautionary measures, with inadequate asepsis, and lumbar punctures in ambulant cases, thus favouring the occurrence of slight inflammatory reactions.

Two cases reported by Forster (*Deutsche med. Woch.*, 1919, No. 33) may here be mentioned, in one of which—a case of latent syphilis—an abortion in the second month occurred after lumbar puncture; and in the other, with a menopause of fourteen months' standing, the menses reappeared after two unsuccessful attempts to perform the puncture. Colleagues have also informed me of single cases of abortion, invariably syphilitic, after lumbar puncture.

CHAPTER IV.

THE MEASUREMENT OF THE PRESSURE.

THE pressure is measured with the aid of the cannula previously described (cf. fig. 3). The patient's head, which has been bent while performing the puncture, should be brought into line with the back, and a few minutes should be waited in order to eliminate accidental factors, such as pressing, mental excitement, &c. Attention should be paid that there are no disturbing external influences; tight underwear at the abdomen, surrounding noises, are sufficient to raise the pressure readings. Then the side branch of the instrument is turned upwards, and a sterile and dry glass tube 20 to 30 cm. long, and with an internal diameter of $2\frac{1}{2}$ to 3 mm., is attached to it by means of a very short piece of rubber tubing. The stopcock is now opened and the spinal fluid will rise in the tube until it has reached a certain level. The difference, measured with a tape or rule, between a line conceived to be horizontal with the puncture spot and the upper level of the column of spinal fluid, gives the height of the pressure in centimetres or millimetres of water. The measurement should not be taken from the end of the cannula, as its position is sometimes oblique. The employment of graduated tubes as recommended by Reichmann is unnecessary. The slight difference between the specific gravity of the fluid and that of water is negligible. Should there be any great fluctuations in the level of the fluid, or if it should gradually sink back, one must wait a little before reading the pressure. A sinking of the level amounting to 10-50 mm. occurs occasionally without any signs of pressing or

mental unrest on the patient's part. Smaller variations of level are quite normal; cardiac impulses transmitted from the cerebral arteries to the brain and thence to the fluid are responsible for variations averaging up to 6 mm. The movements fall in the diastole, not in the systole, owing to the slowness of transmission; they exhibit a simple rise and fall reminiscent of a curve of sines. Their amplitude is greater with higher pressure than with lower, because in the latter case the diminution of space caused by the pulse wave is more readily equilibrated. With greater viscosity of the fluid, due to the presence of blood or pus, their amplitude is less (Becher, *Mitt. aus d. Grenzgeb.*, 35, 1922, p. 343). Respiratory variations in which the rise and fall of pressure correspond to expiration and inspiration occur to the amount of about 20 mm. of water. These variations are often particularly pronounced in cases of injuries to the head and in nervous headache. In rare cases atypical variations are observed, e.g., those observed by Quincke showing a slow rise and fall of the column in intervals of ten to thirty seconds and from ten to thirty mm. in amount. If the fluctuations create any difficulty in reading off the pressure, they can be eliminated by turning the stopcock of the side attachment, after having made sure that the level has already been finally attained. The pressure may prove to be higher than is allowed for by the length of the upright tube; in this case the stopcock of the side branch must be closed before the fluid flows out at the top, and a second tube similar to the first must be joined on by means of a short rubber tube, after which the stopcock may be again opened, the fluid allowed to reach its level and the height read off. In cases of exceptionally increased pressure, as is met with in tumours of the brain, it becomes necessary to join on several such tubes one after another. In every case of lumbar puncture, therefore, several tubes must be kept in readiness.

When the "initial pressure" has been determined one should proceed to carry out the "compression trial" at the neck, or let it be done by an assistant. This consists in applying a moderate pressure to both sides of the neck, with one or two hands, with the object of compressing the jugular veins, which in normal cases, from the veins of the head becoming over-filled, produces an increase of pressure in the spinal fluid. Normally the fluid rises in the tube very rapidly—beginning after fractions of a second—and vigorously, often higher than 100 mm. Care must be taken, of course, that the spinal fluid does not overflow by joining on another length of tube. In the case of space-diminishing lesions within the region of the vertebral canal, such as tumours of the cord (especially of an extra-medullary character), spinal caries, &c., this rise of pressure either does not take place, or it is much retarded and lessened. This is known as Queckenstedt's symptom. The same phenomenon is found to occur in occlusive adhesions due to meningitic lesions (Pappenheim). In order to determine whether the rise of pressure is perhaps due to the psychic influence from the compression of the neck, which is occasionally the case with sensitive patients, I apply a little pressure to the larynx before making the actual trial. Normally, this produces no rise of pressure, of course; and I then soothe the patient with a word or two, if necessary, until the pressure on the larynx produces no effect on the lumbar pressure. If compression on the veins produces no rise, the presence of a mechanical obstruction within the instrument must be excluded, by allowing the patient to cough or press, when even with a positive Queckenstedt's symptom a rise of pressure will follow. With positive Queckenstedt the pulsatory fluctuations in the fluid are absent, but the respiratory variations are maintained, because they are not merely transmitted from the skull, but because the fluctuations of pressure in the thorax are also manifested in the veins of the vertebral canal,

thus producing respiratory waves in the epidural plexus (Becher).

In carrying out the compression trial I make a point of closing the stopcock of the side-branch before relaxing the pressure at the neck, in order to avoid the unnecessary return of the fluid from the vertical tube back again into the lumbar space when the pressure is reduced. I then allow the fluid in the upright tube to run directly into a small test-tube by first opening the principal stopcock, and immediately afterwards opening the stopcock of the side-branch that had been previously shut.

If it is desired to measure the pressure after the withdrawal of a given amount of spinal fluid, which is of importance in lumbar punctures for therapeutic purposes, it is only necessary to close the main stopcock, and to measure the pressure in the manner already described. In all cases the pressure at the end of the operation ("end-pressure") must be determined, because the relationship between the amount of fluid removed and the difference between initial pressure and end-pressure is of interest. Within normal pressure-values, and according to my experience in the withdrawal of small quantities of fluid, the removal of 1 c.c. of fluid corresponds to a reduction in pressure of about 1 cm., though deviations from this often occur, and the pressure usually soon rises again to its original height. A slow diminution of pressure in spite of the withdrawal of large amounts of fluid is found in cases with high pressure and a large amount of fluid. A very rapid diminution of pressure associated with the withdrawal of small amounts of fluid frequently occurs in lesions which occlude the lumbar space. Rapid diminution of pressure in combination with high pressure and the suspicion of cerebral tumour may be taken as a sign of the previously described wedging into the foramen magnum, with a disturbance in the communication of fluid; and this indicates that the puncture must be immediately discontinued, saline solution injected, and the patient placed with the pelvis high and the head low.

The data given in the literature of the subject, with reference to the height of the normal pressure measured in the ascent-tube, show considerable divergence. (The values obtained with the mercury manometer will be dealt with later.) As examples I may mention that Ricker considers a pressure of 40 to 60 mm. to be normal, and one of 150 mm. to be in many cases undoubtedly pathological. Quincke places the upper boundary of the normal at 125 mm. and designates a pressure of 150 mm. as pathological, while Dreyfus does not speak of a raised pressure-value under 200 mm., and Becher has observed in isolated normal cases pressures as high as 220 mm. Weigeldt considers increases of pressure of the fluid only of importance when they amount to 300 mm. of water, in a horizontal position of the body; and upper limits between 170 mm. and 300 mm. are often, he thinks, subject to sources of error; the normal average is 150 mm. to 170 mm. Bárány, on the other hand, finds the "minimal" pressure of the fluid, obtained by allowing the patient to breathe deeply until no further decrease of pressure occurs, to be between 40 and 60 mm. I employed this device long ago, of calming the patient by letting him breathe deeply, but did not examine the matter systematically until Bárány's publication appeared (*Akta Skandinavia*, 1923), after which I found that deep breathing sometimes has no effect on the pressure, and in other cases the pressure is permanently reduced by 20 to 40 mm., provided one has waited long enough for the effect to set in. I cannot regard Bárány's low values as the rule. In normal cases I found pressure-values between 40 and 150 mm., and the majority were higher than 80 mm. Pressures over 200 mm. I regard as pathological (Pappenheim, *Wiener klin. Woch.*, 1924, No. 40).

The highest pathological pressure-values are found in some cases of tumour of the brain and of infectious meningitis—in isolated instances as high as 1,000 mm. of water. Considerable increase of pressure also occurs in

serous meningitis and in hydrocephalus ; also in uræmia and chronic nephritis. Usually moderate increases of pressure are observable in cases of arterio-sclerosis with high blood-pressure, in congestion due to cardiac insufficiency, according to Böttner in polycythæmia (in consequence of increased venous pressure, Weigeldt thinks), in epilepsy, chronic alcoholism, sometimes in alcohol-epilepsy (probably the result of meningeal alterations), and, according to Heine's observations, in various affections of the eyes. In early stages of syphilis there is apparently a usually moderate increase of pressure, which is maintained for a shorter or longer period ; and some writers regard it as a premonitory symptom in syphilitic disease of the approach of inflammation changes in the spinal fluid. In cases of injury to the head, concussion of the brain, gunshot injuries, &c., there is almost invariably in the early stage a considerable increase of pressure due to hæmorrhage and œdema of the brain. In a number of cases an increase of pressure remains, though less pronounced, and it is often a valuable sign of former injury ; this increase of pressure is due to meningeal adhesions which have led to inflammatory irritation of the meninges and to an over-production of fluid. But discretion must be observed in tracing nervous disorders to a cranial injury on the strength of an isolated increase of pressure in the fluid, for any one of the other conditions mentioned, e.g., alcoholism, can be responsible.

It has already been mentioned that numerous external factors, and under normal conditions, can bring about changes of pressure. Thus all kinds of mechanical

¹ Clairmont (*Wien. med. Woch.*, 1923, No. 1) disputes the validity of this statement. He found in a not inconsiderable number of cases of fresh injury to the head that no spinal fluid at all, or a few cubic centimetres, or only a few drops of blood-stained spinal fluid escaped, and no increase of pressure was noted. He regards this as a consequence of a disturbance of secretion of spinal fluid, and thus as the expression of a most severe injury, and of unfavourable prognostic significance; and it is probably due to traumatic changes in the brain, leading to an increase in its volume (œdema of the brain?).

influences, the action of muscles, coughing, pressing, the pressure from a tight garment, pressure of the hand on the abdomen, may lead to an increase of pressure. Even differences in the position of the head while performing the puncture can lead to a rise of pressure¹; and more especially is this the case when the patient is sitting. One should therefore see that during the measurement of pressure the head is always aligned with the trunk. Mental excitement, fear, disturbing sense-impressions, &c., can act in a similar manner. On the other hand, it is possible to obtain erroneously small pressure-values when the spinal fluid leaks around the needle into the adjoining tissues ("Fistelung")—though such an occurrence must be rather rare—or when there is leakage of fluid at any point.

¹ According to Count Haller this results from variations in the size of the cisterna magna due to tension or relaxation of the occipito-atloid and the atlanto-axial ligaments. Haller concludes that in this manner nodding movements of the head exercise a sort of pump action, causing a downward propulsion of fluid when the cisterna is reduced in size, and, as no fluid could return into the cisterna through the narrow posterior part of the subarachnoid space, an aspiration of fluid out of the ventricle when the cisterna is enlarged. This view is unproven, and, I think, under normal circumstances, highly improbable. There is, in my opinion, a much greater plausibility for the view that the fluid circulates back and forth between the cisterna and the upper part of the spinal subarachnoid space.

CHAPTER V.

THE SOURCES OF ERROR IN PRESSURE MEASUREMENT. OTHER METHODS OF MEASURING THE PRESSURE.

SOME writers hold that the pressure need not be measured, because sufficient information of the prevailing conditions of pressure can be obtained from the manner in which the fluid escapes from the needle—slowly dripping, rapidly dripping, flowing in a jet, &c. I do not think this correct, because, although it is possible in a large number of cases to form a fairly accurate opinion, it is at times impossible to avoid mistakes. A slight misplacement of the needle, for instance, can occasion the fluid to escape in drops, in spite of an increased pressure. Or conversely, owing to mental excitement of the patient, the fluid can flow out in a stream, notwithstanding normal pressure—a circumstance that can be much more readily interpreted from the slow sinking of the column of fluid than by observing the rate of escape without measuring the pressure. If, however, it is desired to estimate the pressure without measuring it, one must not forget to wait patiently for a few minutes before allowing the fluid to escape, in order to exclude as far as possible the incidental factors. I would not care to leave out the measurement of the pressure, which is very simple if performed as here described, inasmuch as I consider it of importance to keep the pressure under observation during the puncture, even when it is only for diagnostic, and not for therapeutical purposes. In some cases, as e.g., when there is but a remote suspicion of tumour of the brain, the measurement is indispensable.

Some of the methods in use are rather complicated, which accounts to some extent for the aversion against measuring the pressure. A further reason is no doubt due to the fact that in measuring the pressure there are various sources of error inherent in the method itself, which, unlike the accidental factors mentioned above, cannot be eliminated. First of all there is the error that in lumbar puncture it is not the actual conditions of pressure that are investigated. By opening up the dural sac an alteration in the conditions of pressure is created which it is impossible rightly to estimate. It has not hitherto been possible to exclude this source of error, which inheres, of course, just as much to the method of estimating the pressure without measurement. It would only be possible to eliminate it if one could apply a pelotte (*sic*) to the uninjured dural sac, and then measure the pressure. A further source of error is contained in the upright tube method. It consists in the fact that the pressure is measured by the height of the escaping column of fluid, i.e., that the pressure is not measured until the spinal fluid that is to indicate the pressure has left the body. This contains indubitably a certain amount of inaccuracy, which is, moreover, not common to all cases, as the diminution of pressure does not always run parallel to the amount of fluid withdrawn.¹ For instance, a pressure-value of 200 mm., measured by the method here described, does not show the pressure of the fluid as it was in the original condition in the body, but only a pressure of the fluid less the volume contained within the puncturing apparatus. This volume, in a tube of 3 mm. internal diameter, amounts to $r^2\pi h = 1.5^2 \times 3.14 \times 200 = 1,413$ c.mm., to which must be added the small amount of fluid in the cannula and the connecting tube, say $1\frac{1}{2}$ c.c.

¹ Comparative researches by Kausch's method (cf. close of the present chapter) have shown me, however, that this error is minimal.

For this reason Krönig has employed capillary tubes of 1 mm. in diameter for the ascending-tubes; but my experience of them does not incline me to favour their use, owing to the powerful capillary action of such tubes, and the disturbing influence of air bubbles which in wider tubes can be easily removed by tapping the side. I prefer, therefore, somewhat wider tubes. Krönig's apparatus consists, for the rest, of a simple cannula provided with a stopcock, which is connected by means of a short rubber tube to a tube bent at a right angle. The upright tube is attached to the vertical limb of this tube with a piece of rubber tubing. The horizontal limb possesses a branch which is directed downwards, and carries a piece of rubber tubing and a pinchcock; fluid can be allowed to escape from this during the puncture. By simplification of this outfit I arrived at the method of measuring the pressure herein described, by substituting a side branch for the middle limb, which renders Krönig's instrument very complicated.

Quinke, the originator of lumbar puncture, also described an apparatus for the measurement of pressure. He made use of a long rubber tube provided at one end with a cone that fitted into the end of the cannula. The rubber tube served partly as ascending-tube, but it had at its other end a glass tube in addition. In measuring the pressure the cone of the rubber tube was placed into the end of the cannula and the rubber tube held horizontally, raising it gradually until the spinal fluid appeared in the glass tube, when the height of the column of fluid was measured. When employing this outfit, it is necessary after removing the stylet to close the cannula with the thumb so as to avoid loss of fluid. This, however, does not always succeed, and the asepsis is, moreover, impaired. These disadvantages induced Krönig to adapt a stopcock to his cannula. A further disadvantage attached to Quinke's method is that the rubber tube contains a considerable amount of fluid that is not taken into account

in measuring pressure, which increases the previously described errors—a disadvantage which is lessened by the employment of Krönig's attachment, and still more so with a side branch, as in my method. The use of long rubber tubes is also inexpedient because their elasticity soon changes, which renders the results of the pressure measurement unequal and necessitates frequent renewal of the tube.

The measurement of pressure by means of a mercury manometer was first recommended by Krönig, and afterwards by Wilms and Bungart. The last-named writer uses a glass U-tube mounted on a wooden stand. The U-tube is bent horizontally at one end, to which is attached a piece of rubber tube connected with the puncture needle. The U-tube is filled up to a certain level with mercury, and there is a millimetre scale between the two limbs. The wooden stand is provided with a spirit-level so as to enable the vertical position of the instrument to be kept under control. Bungart employs a cannula with a three-way stopcock; but the cannula with stylet already described can be used instead.

Bungart finds in the lateral position normal pressure values of 6 to 20 mm. mercury, and in the sitting posture from 20 to 35 mm. Hg; but he stresses the view that the pressures obtained in the sitting posture are the more constant and significant. This is probably because in the lateral position the low pressure on the column of mercury, with its readings thirteen times smaller than those of a column of water, gives less distinct readings—a circumstance which is perhaps not void of significance in interpreting the compression trial at the neck. Bungart's objection to the upright tube method, viz., that with high pressure the fluid jets out, is overcome by adopting the technique I have described. An advantage claimed by Bungart for the mercury manometer is that it enables negative pressure to be measured, which is of importance when undertaking punctures of the brain.

The outflowing fluid, moreover, is not employed in the measurement, or only to a minimum amount, which is a theoretical advantage in its favour.

Measurement by means of an aneroid manometer, which has been recommended by Claude, Porak and Rouillard, possesses the same advantage. These writers use an aneroid which is graduated to centimetres of water, and is connected with the cannula by means of a long flexible pipe that is interrupted at intervals by small glass tubes. Longer glass tubes with small connecting pipes are more practical, and the cannula with stylet is to be preferred. These writers find the normal pressure to amount to 12 to 15 cm. of water, but also to 18 to 20 cm. of water. A probable disadvantage of the method is in the fact that aneroids easily get out of order, and therefore need frequent testing.

The error which results from measuring the outflowing fluid can be avoided, as Kausch suggested, by reading the height of the pressure, not of the rising spinal fluid, but of a liquid which opposes the outflow of spinal fluid and corresponds to its pressure. In Kausch's apparatus a short glass tube with a mark at equal distances from the ends is connected by a long pipe to the cannula. The cannula possesses two branches, one of which has a capillary lumen, while the other has a lumen $3\frac{1}{2}$ mm. in diameter; the stopcocks of both branches must be closed. Before performing the puncture the pipe with its glass tube is attached to the wider branch of the needle, with closed stopcock, and filled up with sterile salt solution. The stopcock is then opened and salt solution is allowed to escape until the upper level reaches the line in the glass tube, when the stopcock is closed again. The stylet, which has been previously removed, must now be introduced again and the puncture performed. As soon as the point of the cannula is in the subarachnoid space the stylet is withdrawn, but not entirely—the needle is so constructed that fluid is able to flow into the side branches

without completely removing the stylet—and the stop-cock of the branch with the wide lumen is opened. The spinal fluid now presses against the salt solution contained in the tube, which is then moved in such a manner that the level again reaches the line in the glass tube, after which the difference of level is measured in the customary manner. The capillary branch of the cannula serves the purpose of collecting spinal fluid during the operation.

Apart from its complication, this ingenious method possesses the disadvantage that it employs a tube with variable elasticity; and there is further an admixture of salt solution with the spinal fluid at the point where they meet, which interferes with the accuracy of investigations of the fluid.

CHAPTER VI.

THEORETICAL CONSIDERATION OF PRESSURE
MEASUREMENT.¹

THE physiological and pathological conditions governing the pressure of the spinal fluid are very manifold, and to some extent their essential nature is still obscure. In the literature of the subject, erroneous views of the physical factors that determine the height of pressure of the fluid in the sitting posture are also somewhat prevalent, and from the viewpoint of the history of science research this aspect of the literature is not without interest.

A work that is cited as of fundamental importance upon this problem is that of Grashey, which appeared in 1892 in the form of an essay in honour of Buchner. Later writers on the subject, however, have not bestowed the attention that this admirable, penetrating, and consistently executed work deserves. Grashey's researches are usually summarized as follows. Grashey drew attention to the fact that differences prevail in the physical conditions in the cranial and spinal cavities respectively. The dura mater of the cranium everywhere forms an adherent lining to the bony cavity, while in the vertebral canal there is the epidural space, which is chiefly filled by the plexus of valveless veins, which, arranged in elongated meshes and connected transversely, surround the dural

¹ My grateful thanks are due to Dr. Carl Horowitz of the First Institute of Physics, Vienna, and Dr. Alexander Spitzer, of the Neurological Institute at the University of Vienna, for their valuable assistance during the study of this problem.

sac on all sides, investing it with a liquid mantle. Owing to these differences, the physical conditions are as though the spinal portion of the dural sac, as far as its entry into the skull, were contained within a rigid vessel filled with liquid, into which another vessel with rigid walls, and a downwardly directed opening, to wit, the skull, is immersed. In such an arrangement the zero point, i.e., the point at which the pressure is equal to atmospheric pressure, lies at the boundary line between the two vessels, in this case, and with reference to the cranio-vertebral cavity, in the neighbourhood of the foramen magnum. The hydrostatic pressure at the base of the venous column of liquid, as well as in the column of spinal fluid which it surrounds, is therefore equal, in the erect posture, to the height of this column of liquid; or the distance between the lower end of the lumbar space and the foramen magnum, about 60 centimetres; while at the summit the pressure is negative and corresponds to the distance from the foramen magnum—about 13 centimetres.¹ The pressure at the side walls of the dura is equal to zero, for the conditions of pressure on both sides are equal, and for the same reason the pressure there is not altered when the position of the body is changed. On the other hand, the hydrostatic pressure undergoes a change when the body is inverted, for now the pressure of the column of liquid in the vertebral canal is augmented by that corresponding to the height of the cranium, and the pressure at the [cranial] summit would be $60 + 13$ centimetres.

The investigations of Krönig and Gauss in the case of living persons, and their researches conducted on the calf and on the cadaver, show that Grashey's conclusions

¹ I follow the usage in the literature of the subject in designating a pressure smaller than that of the atmosphere as negative; but I desire to guard against the mistaken view, occasionally expressed, which represents a liquid in a state of "negative pressure" to be, as it were, subject to a pull.

do not agree with the actual circumstances. When a puncture is performed in the sitting posture, the hydrostatic pressure is by no means as great as the height of a column of liquid between the foramen magnum and the site of the puncture. The measurement of pressure in the sitting posture shows rather that the height of the column of fluid in the ascending tube does not exceed that of the upper thoracic or the lower cervical region of the cord, and that when a higher spot is punctured no fluid is obtained.¹ In the cadaver it was found, moreover, that in the inverted position a much lower pressure was obtained than that to be expected from Grashey's conclusions. And as both investigators were able to show that the fluid in the ascent tube behaved in the same way as in communicating tubes, i.e., by puncturing at different levels the column of fluid invariably rose to the same height, they assumed that in the erect posture the column of fluid does not extend higher than the region of the upper thoracic cord, and that above the zero point in the region of the cord the arachnoid and pia are so close together as to contain just so much fluid as to moisten the interstitial walls. From this they concluded that when the position of the body is changed considerably, as when inverted, or in a highly inclined plane with raised pelvis, the whole column of fluid undergoes a displacement from the lower part of the trunk towards the cervical region of the cord.

As opposed to this, Propping now submitted that in the position with raised pelvis it was possible to aspirate fluid from the lumbar region by puncturing into a part of the subarachnoid space in which, on the assumptions

¹ In measuring the pressure by means of the upright tube a somewhat lower pressure is manifested owing to the outflowing fluid being itself employed in the measurement. On the other hand, in the case of puncture in the sitting posture, account must be taken of the not inconsiderable components of vital pressure which are added to the hydrostatic pressure. (The latter also occur in the case of puncture in the lateral position.)

of Krönig and Gauss, in this position no fluid should be present; and that therefore the conclusion drawn by Kronig and Gauss is unjustified, viz., that no spinal fluid is present above the zero point as determined by the method of the upright tube.¹

Propping advanced, therefore, another explanation to account for the conditions of pressure found by Krönig and Gauss. He considered that Grashey's view that the cranium resembles a vessel with rigid walls is indeed correct; but that the epidural space, on the other hand, cannot be regarded as a rigid vessel, as Grashey thought. In consequence of the loose fatty tissue which fills a part of the epidural space, and owing to the fatty tissue being pressed, when the dura is distended, into the many lacunar spaces within the vertebral canal, the epidural space constitutes a wall possessing a certain elasticity.

Propping then devised a physical experiment under conditions which corresponded to his view of those obtaining in the body. He took a test-tube to represent the rigid-walled vessel, or skull, and joined it to a rubber bag, which was intended to correspond to the elastic wall in the vertebral canal. Just so much water was introduced so that no over-pressure was present when the tube and rubber bag were placed in a horizontal position. When the position was then changed to the perpendicular, the lower part of the rubber bag bulged out, and the upper part appeared to be pressed inwards, while the zero-point sank below the boundary between the test-tube and the rubber bag. Accordingly the pressure at the base of the rubber bag was less than that of a column of liquid as high as the total length of the bag. Propping considered that in the erect posture there must occur a similar distension of the lower part

¹ This conclusion, however, is only partly justified, as Walter was able to show that by the same method it is possible to aspirate fluid from a still lower region of the arachnoidal sac.

of the dural sac and a similar lowering of the level of zero pressure. He thus also finds that there is a displacement of the subarachnoidal fluid when the position of the body is changed, but he describes this displacement as "relatively small." With reference to the displaced fluid—upon the amount of which no light is thrown by measuring the pressure, as Propping rightly observes—it would appear to make little difference whether one assumes with Krönig and Gauss that in the erect posture the whole of the fluid undergoes a downward displacement, or with Propping that a thin layer of fluid still remains in the upper parts.

Propping further demonstrated the existence of a negative pressure in the cervical region by experiment and in the cadaver,¹ and showed that on puncturing in this region air entered with a hissing sound. Walter afterwards extended Propping's researches, and measured the negative pressure existing above the zero-level by physical experiment, and post mortem. He established that its height varied according to the distance between the zero-level and the boundary between the rigid and the elastic parts of the "system"; i.e., that at a given point the [negative] pressure is greater the deeper the zero-level sinks in the system.

Now, the experiments carried out by Propping and Walter are defective, in that they leave out of account the importance of the vascular system, which had already been duly appreciated by Grashey, who, moreover, had already performed the same experiments as they, and in a much more perfect manner, by means of an ingeniously constructed apparatus. Although a full account cannot be given here of his experiments and reflections, which

¹ It must be remarked, however, that the greatly altered conditions after death do not permit of the unreserved application of the findings to the living human being, for it is not impossible that after death the fluid really sinks downwards in the way Propping conceives, which is not necessarily the case during life.

progressed by degrees from simple to increasingly complicated conditions; yet it will be necessary to deal with one or two points in the physics of the subject, keeping mainly to Grashey's own description.

If a tube which is closed at the ends by elastic membranes is filled with water so that the membranes do not bulge out—(Grashey accomplished this by adjusting to each membrane a metal cap provided with a very small opening to enable air to escape)—and the tube is then placed in a perpendicular position, the result is that the lower membrane is depressed outwards, and the upper one inwards. Within the tube the upper membrane is thus subject to a negative pressure and the lower membrane to a positive pressure. In the tube the pressure is partly negative and partly positive, while that prevailing at the border of these regions of pressure is zero. Given an equal distensibility of the membranes the zero-level is at the middle of the tube,¹ while the pressure below the middle is positive, that above it negative. (Grashev measured very accurately the pressures prevailing, negative as well as positive, in different parts of the liquid, and had thus already established the facts observed later by Propping and Walter in their experiments.) If the two membranes are of unequal stretching capacity ("Dehnbarkeit") the limit of the regions of pressure is displaced higher the more the upper membrane is capable of

¹ The explanation of this fact, which Grashey does not give, is as follows: The column of liquid in the vessel is not subject to the whole atmospheric pressure, as a part of this is taken up by the membranes. The latter, however, become stretched until they are both in equilibrium with the weight of the column of liquid, i.e. (on the assumption that the membranes are equal)—until $2e = \phi$, in which e is the elastic alteration ("Nachgiebigkeit") of one of the membranes, and ϕ is the weight of the column of liquid. The pressure at each part of the column of liquid is therefore the atmospheric pressure (A) diminished by the elastic alteration of one of the membranes, and augmented by the weight of the superposed column of liquid (π)—a pressure, therefore of $A - e + \pi$. The zero-level is thus to be found where $e = \pi$, i.e., however, where $\pi = \frac{\phi}{2}$, or in the middle of the whole column of liquid.

stretching, and conversely ; until finally, when the upper membrane has arrived at infinite stretching capacity, i.e., offers no further resistance ("unendlich elastisch wird, d. h. gar kein Widerstand mehr leistet"), the limit of the pressure regions ascends to the uppermost level of the tube as if there were no upper membrane, i.e., everywhere positive pressure. If, on the other hand, the upper membrane is of indefinitely small "elasticity," i.e., perfectly rigid ("unendlich wenig elastisch, d. h. vollständig starr"), the boundary of the regions of pressure lies in the lowest level of the tube, and the lower membrane is free from pressure of the column of water, provided the latter does not exceed 34 feet in height.¹

Grashey examines further the conditions in rigid tubes which in addition to possessing elastic membranes at both ends are also provided with elastic membranes at the side walls ; and, finally, the conditions when the whole side wall of the tube is elastic in all transverse sections, which is the case when an elastic tube is employed. If the tube is equally distensible in all transverse sections, the boundary of the pressure regions lies in the middle ; and if the lower parts of the tube are more distensible than the upper, the boundary is displaced downwards and conversely. When the walls of the elastic tube are very thin without being very elastic, i.e., with small elastic change to positive pressure and unusually small resistance ("ungewöhnlich stark nachgiebig") negatively, in the sense that the tube collapses until the walls touch, then the boundary of the pressure regions, under the given conditions of the experiment, is very high, because the negative alterability far exceeds the

¹ Grashey uses the expressions "distensible" ("dehnbar") and "elastic," as well as "of infinitely small elasticity," and "rigid," as synonymous, which is incorrect. I have kept to Grashey's description as literally as possible, in spite of its being difficult to follow, and perhaps misleading in places, because the difficulties mentioned may be overcome with a little attention, when the accuracy of his conclusions will be evident.

positive, and because the upper parts of the tube become subject to negative pressure, the lower parts to positive.¹

The results of these physical experiments Grashey applies to the cranio-vertebral cavity as follows: If the cranium is regarded for the time being as a rigid-walled receptacle continued into the rigid-walled vertebral cavity that is elastically closed only below by the dura mater, then the effect of the vascular system of the cranium, the main branches of which are at the base, is that of an elastic membrane which is attached to the side walls of this rigid system at the level of the base of the skull. As the distensibility of the lower closing membrane, the dura mater, in proportion to that of the cranial vascular system is infinitesimal, the boundary of the regions of positive and negative pressure lies at the level of the upper membrane, i.e., at the base of the skull, and above it the pressure becomes increasingly negative towards the vault of the skull, and below that level increasingly positive up to the end of the vertebral canal.

Now, the vertebral canal also contains blood-vessels, and the question arises as to what the effect is of this vascular system on the conditions of pressure in the cranio-vertebral cavity. Unlike the cranial vascular system, the spinal vascular system is not a closed system of distribution ("in sich geschlossen")² but at different levels of the vertebral cavity arterial branches enter, and venous branches leave, traversing the dura mater. Their effect is equal to that of elastic membranes set in the side walls at the level of their points of entrance and

¹ In other words: If a membrane is more collapsible ("einfaltbar") and less distensible ("ausdehnbar") it will more readily collapse in the upper, or region of negative pressure, than it will distend in the lower, or region of positive pressure. The conditions are thus analogous to those in which the upper closing membrane is more distensible ("dehnbarer") than the lower, in which conditions the zero-level also rises.

² By this Grashey means the entrance and exit of the arterial and venous systems at the same height of transverse section.

exit, and possessing the same distensibility as the vessels. As they are within the region of positive pressure they tend to displace the boundary of the pressure downwards. Grashey, referring to the literature, then points out that the numerical proportion of spinal vessels to intracranial is indefinitely small, and that therefore the distensibility of the former as against that of the latter is negligible. He is thus of the opinion that the boundary of the regions of pressure, notwithstanding the existence of the spinal vascular system, must be either at the level of the base of the skull or only very little below that point.

Grashey then discusses the fact that the spinal space occupied by fluid is not only closed elastically at its lower end, but is invested on all sides by the dural sac. (He stresses the fact that the vertebral canal has no rigid side walls.) As the tissue of which the dural sac is composed is very tense, and very little distensible in the positive direction, yet easily collapsible; while in the negative direction its distensibility is relatively great, the effect of the conditions of distension of the isolated dural sac is, according to the preceding remarks, that the boundary of the pressure regions is very high.

The circumstance, finally, that the dural sac is not isolated, but is in a hollow cylinder filled with venous blood within the bony vertebral cavity, does not alter the hydrostatic conditions described; its only effect is that the pressure in the dural sac and that in the hollow cylinder surrounding it, formed by the spinal vein plexus, remain equal in amount.

In this manner Grashey arrived at the statement which is cited in the literature of the subject, that in the erect posture the zero level of hydrostatic pressure is in the neighbourhood of the foramen magnum. Though this assumption is not in accordance with the facts, the actual zero level being lower down, it should not be overlooked that Grashey in his researches aimed at showing that the

zero point was not at the cranial summit, but under no circumstances higher than the base of the skull ; and that the fact discovered later that the zero point lies still lower down, is not a refutation, but only an extension of his views. Had Grashey known the actual circumstances—we must remember that his work appeared quite shortly after Quincke's introduction of lumbar puncture—his own researches would have enabled him to explain this fact.

It would appear that Grashey's assumption that the vascular system of the cord is so much less considerable than that of the brain that its elasticity is negligible, is not anatomically justified. If, however, it is assumed in opposition to Grashey, that the elasticity of the spinal vascular system is not negligible, then Grashey's experiments and conclusions *demand* the placing of the zero level lower down. His view that the elasticity of the vascular system takes up a part of the hydrostatic pressure distinguishes it essentially from that of Propping, who attributes the diminution of pressure to a shortening of the column of spinal fluid in the spinal cavity.¹ Another factor not mentioned by Grashey may be considered to produce a similar effect. The spinal space occupied by fluid is in places only interstitial, which involves the existence of a capillary attraction that opposes the hydrostatic pressure, thus diminishing it. We know, however, just as little of the extent of this capillary action as we do of the extent to which the spinal vascular system is capable of modifying the hydrostatic pressure.

¹ It is also clearly not justifiable to regard the epidural space as equivalent to the atmosphere, on which assumption the experiments of Propping and Walter are based. If Propping's apparatus is placed in a vessel containing liquid, no change of form takes place in the rubber bag.

Gerstenberg and Hein (*Zeitschr. f. Geb.*, 61, 1908, p. 524) also pointed out the significance of the vein plexus in the displacement of spinal fluid ; and Propping's view that the epidural fatty tissue plays a part in the displacement of fluid is contested by Count Haller, Becher, Weigeldt and others. But Grashey's researches have hitherto never been correctly cited and adequately estimated.

The conclusion which Grashey drew, that there is a negative pressure in the skull (provided there is no pathological increase of pressure) does not seem to me to give rise to any special difficulties, as the pressure values concerned are comparatively small.¹ Propping's assumption that in walking about an increased secretion from the plexus takes place, bringing the pressure up to that obtaining in the sinuses, is unnecessary, for, as Walter rightly replies, a lumbar puncture performed after the patient has walked about considerably, ought to show higher pressure-values than one carried out after lying for a long time. Grashey established further, from theoretical considerations, that when the cranial cavity is opened, including the dura mater, the pressure conditions are essentially different from those obtaining when the cranium is intact; the negative pressure in the skull then disappears and a positive pressure results, which becomes added to that prevailing in the vertebral canal. Comparative investigations of the pressure before and after trepanation might be of value in settling this question.²

The discussion of the components of hydrostatic pressure when puncturing in the sitting posture shows that they depend upon factors of which an exact knowledge is lacking. It is thus not surprising that these components can exhibit very considerable variations. The pressure in the sitting posture is normally from 200 to 300 mm. of water higher than in the lateral position, which gives quite a large physiological range. For this reason it is in general not advisable to measure the pressure in the sitting posture; this should be carried

¹ Eskuchen found a negative pressure amounting to a maximum of 150 mm. of water by punctures performed on the cisterna in the sitting posture. (Cf. Appendix.)

² Weigeldt mentions in this respect that "Measured in the erect posture and in the lumbar region, the pressure of the spinal fluid after trepanation is . . . immediately raised. The equalization of pressure is almost instantaneous."

out only in the lateral position, in which only those factors are expressed with the investigation of which we are concerned in pathological cases, viz., more or less distension of the lumbar sac, tension of the surrounding tissues, state of distension of the ventricles of the brain and of the vessels; these are the factors in producing the pressure which, at the suggestion of Quincke, and in contradistinction to hydrostatic pressure, are called "vital pressure."¹

Only in one group of cases does it appear preferable, according to Bungart's investigations, to carry out the measurement in the sitting posture, namely, in cases of injury to the skull. In such cases, Bungart found that a pathological increase of pressure when puncturing in the lateral position is absent, except in fresh cases, while it may be observed when puncturing in the sitting posture. Bungart accounts for this circumstance, apparently with reason, by supposing that when puncturing in the lying posture, slight adhesions of the meninges prevent the over-pressure present in the brain from finding expression at the distant site of puncture; while in the sitting posture the whole column of fluid is effective and is able to overcome this obstacle.

¹ The unintelligible observations of Bungart, to the effect that in puncturing in the lateral position the pressure of the spinal fluid is greater the lower down in the vertebral canal the puncture is performed, have been disposed of by the investigations of Weigeldt and Eskuchen. Weigeldt found in the majority of cases in the lateral position that the pressure in the ventricles was somewhat higher than in the subarachnoidal space, which may have been secretion pressure. Eskuchen found exactly the same pressure in the cerebellar cistern as in the subarachnoid space.

CHAPTER VII.

PHYSICAL CHARACTERS OF THE CEREBRO-SPINAL FLUID.

ADMIXTURE OF BLOOD, XANTHOCHROMIA, MICRO-ORGANISMS, CHEMICAL CONSTITUTION.

THE normal cerebro-spinal fluid is a clear, watery fluid with a specific gravity of 1·0065 to 1·0073, and with only a slightly higher viscosity—1·01 to 1·06—than water, and a slightly alkaline reaction.¹ In pathological cases with considerable increase of cells—beyond about 700 [per c.mm.]—the fluid assumes an appearance resembling that of sun-motes; in meningitis it has a dull, milky appearance.

The normal fluid contains no red blood-corpuscles. An admixture of blood is therefore either artificial or pathological, and is attributable in the former case to an injury to a small blood-vessel during the puncture, in the latter case to hæmorrhages in the delicate membranes of the brain, or in the central nervous system, or in the dura with a passage of blood into the subarachnoid space, as in aneurysms, after traumatism of the skull or the cord, in epilepsy, eclampsia, arterio-sclerosis, encephalitis, malaria, purpura, hæmorrhagic internal pachymeningitis, acute meningitis, &c.

¹ Ylppö (*Zschr. f. Khk.*, 1918, vol. 17) found from researches in children that with sensorial dullness the alkalescence diminishes, and more so in cases of acute disease than in chronic cases (pH = 7·18, as against 7·78 normally). In disturbances of consciousness due to non-cerebral diseases the alkalescence is normal.

The differentiation of an artificial admixture of blood from one due to a pathological lesion is usually easy from certain characters which distinguish recent bleeding (artificial hæmorrhage is, of course, always recent) from older hæmorrhages (at the time of the puncture in pathological cases they are generally old). In recent cases of pathological hæmorrhage, which are very rarely punctured, the criteria are often doubtful. In recent bleeding the fluid is cloudy owing to the suspension of red blood-corpuscles; in older hæmorrhages the fluid is clear, the corpuscles being dissolved. If a specimen of spinal fluid containing fresh blood admixture is centrifuged it becomes colourless, or at most it retains a pink shade; in older cases of bleeding the centrifuged spinal fluid has a yellowish or reddish colour. The latter is due to the presence of hæmoglobin and indicates a rapid dissolution of the red corpuscles (*tachémolyse*), while the yellowish colour is from derivatives of hæmoglobin and indicates a slow dissolution (*bradémolyse*, Anglada). Similarly in fresh bleeding spectroscopic examination sometimes shows the oxyhæmoglobin bands, while in older hæmorrhages there is usually no characteristic spectrum. A recent hæmorrhage tends to the formation of clots, in contradistinction to essential hæmorrhage, in which by the coagulation *in loco* the fibrinogen is, according to Mestrezat, to some extent removed.

The circumstance that pathological hæmorrhages are more regular, while artificial hæmorrhages are often irregular, can, up to a certain degree of probability, be taken advantage of in differentiating between an artificial and a pathological fresh hæmorrhage; and it may be regarded as a decisive sign of an artificial hæmorrhage, which much more frequently occurs than a fresh pathological hæmorrhage, if the first admixture of blood with the fluid disappears or becomes appreciably less. In pathological cases, e.g., in apoplexy, in hæmorrhagic internal pachymeningitis, red blood-corpuscles, or blood

pigment derivatives, are sometimes only found after the removal of large amounts of fluid.

In older hæmorrhages altered erythrocytes are found, also hæmoglobin derivatives, blood pigment, flakes of hæmosiderin, both inside and outside of cells; also the secondary reaction-pleocytosis, by which there is an increase of cells a day or two after such hæmorrhages; these all vary considerably according to the extent of hæmorrhage, to the lapse of time between the hæmorrhage and the first investigation; but above all according to whether the hæmorrhage has occurred only once or at successive intervals. At first, chiefly lymphocytes and polynuclear leucocytes are found, to which at a later stage macrophages are added (*see* Chap. X). The disappearance of the polynuclear cells betokens the arrest of the hæmorrhage; increase of the lymphocytes can sometimes be present longer than the yellow coloration of the fluid. In the case of brief hæmorrhages the reaction signs in respect of the cells can be absent, and there may be no more than a yellow coloration of the fluid, so-called xanthochromia. All the symptoms, however, of an older pathological hæmorrhage can be present if an artificial hæmorrhage has been brought about some time previously by a lumbar puncture, and has led to a local pathological reaction of the meninges.

Yellow coloration, or xanthochromia, in which the spinal fluid is often strongly tinged an amber or lemon yellow colour, occurs after extensive hæmorrhages, and also in various pathological conditions in which the presence of red blood-corpuscles in the fluid is not demonstrable. In cases of occlusive lesions in the vertebral canal it is sometimes very pronounced, especially in extramedullary tumours. It is frequent, though usually less pronounced, in all possible forms of meningitis. Xanthochromia has also been observed in cardiac insufficiency and in general diseases, as well as in carbonic oxide poisoning and in the convul-

sive attacks of the early stages of syphilis. Lange emphasizes its occurrence in tumor cerebri and considers it of differential value in diagnosing doubtful cases of this disease. As a rule in such cases the coloration is of small degree, being merely the expression of minimal hæmorrhages; and the same observation can be made with regard to hæmorrhagic internal pachymeningitis. Special methods are necessary to establish it. If looking through the depth of the fluid on a white ground is insufficient, a layer as deep as possible should be placed in a colorimeter vessel and compared with distilled water, after having vigorously centrifuged the fluid. According to Zaloziecki the spinal fluid of paralysis also sometimes shows in a very deep layer a faint yellowish coloration.

In the majority of cases in which xanthochromia occurs, it is caused by multiple hæmorrhages, which may be but slight, and which lead to the formation of bilirubin.¹ In cases of congestion of spinal fluid due to a space-diminishing lesion, and perhaps in some cases of meningitis, it arises from the passage of 'colouring matter from the plasma into the spinal fluid.' Jáundice of long duration causes a yellow coloration of the fluid originating from bile pigments, and in deep layers it is not infrequently greenish.

In infectious diseases of the central nervous system micro-organisms are found in the spinal fluid; their presence may be established by various methods, among which the following may be briefly mentioned:—

(1) The fluid is centrifuged and the sediment is smeared on a slide or a cover-glass. The preparation is then dried, drawn through the flame, and stained with Löffler's methylene-blue, or by A. Pappenheim's method, or

¹ The change into bilirubin is said to be brought about by the vital activity of the endothelial cells. Three days after the injection of blood into the lumbar sac, Leschke found xanthochromia and characteristic reactions of bile pigments; while this change could not be obtained in a test-tube (*Deutsche med. Woch.*, 1921, No. 14).

Gram's, &c., or for the presence of tubercle bacilli by Ziehl-Neelsen's method, which may be also employed with Gram-stained preparations. Fibrin clots are often specially suitable for establishing the presence of tubercle bacilli. The clots are lifted carefully with a platinum needle and spread out as flat as possible on a slide. A previous enrichment of the fluid by incubating at 37° C. for a day or more is often to be recommended. Lange recommends enrichment in 1 to 2 per cent. sugar-broth, or in Besredka's egg-broth, or in a liquid medium composed of urine with an addition of 1 to 2 per cent. of Witte's peptone, dextrose, and peptose.

(2) The culture method: The spinal fluid is allowed to run from the cannula on to an agar or blood-agar plate and after incubation the cultures are examined for the culture-features, and microscopically in smear preparations; also by inoculation on various culture-media.

(3) Animal inoculation, by which means it is possible to transfer tuberculosis, syphilis, and more recently epidemic encephalitis, and possibly also multiple sclerosis.

Practically all micro-organisms which otherwise produce pathological effects in the body come into consideration as exciting causes of meningeal diseases. In some cases of general infection the specific organisms, e.g., diphtheria and typhus bacilli, have been found in the spinal fluid, and without any signs of meningeal inflammation either in the clinical picture or in the fluid. (Great reserve is necessary in interpreting the findings, both positive and negative, in bacteriological examinations of the spinal fluid, and only repeated, exact investigations should be judged upon, for the establishment of micro-organisms is often difficult, and contaminations are also liable to be present.) *Spirochaeta pallida* has also been repeatedly discovered in the fluid, and before the appearance in it of any inflammatory changes of a syphilitic character. The fluid has, moreover, at times been found to contain cysticercus vesicles, membranes and hooks of echinococcus, actinomyces granules, &c.

The references to the chemical constitution of the spinal fluid will here be limited to a few data. Chemical examinations of the fluid are not without difficulties, owing to the small amounts at our disposal ; but some progress has resulted from the recent adoption of micro-methods of investigation.

The spinal fluid contains nearly 99 per cent. of water. Of the remaining 1 per cent. or more, three-quarters consist of mineral substance, and a quarter of organic matter. Sodium chloride forms the bulk of the salts, of which in normal fluid from 7.25 to 7.5 per thousand parts is present (Lange gives 7.3 to 7.35 per thousand) ; thus in a higher state of concentration than in average blood-serum, in which the amount of sodium chloride is 5 to 6 per thousand parts. An explanation of this fact has been sought in the low osmotic pressure of the small amount of albumin in the spinal fluid, which necessitates an increase of pressure for the preservation of equilibrium in the fluid, which depends chiefly on the amount of sodium chloride. An increase of chlorides in the spinal fluid occurs with increased osmotic pressure in the blood, e.g., in renal insufficiency, and, according to Depisch and Richter, in hypertonia. A decrease of chlorides is found accompanying an increase of permeability, which reaches its highest degree in tubercular meningitis, and in this disease attains diagnostic value. In cases with marked signs of inflammation, and a distinctly progressive character of the disease with simultaneous decrease of chlorides in the blood, it can be decreased to 6 to 5 per thousand parts, while the amount found in pyogenic meningitis does not sink below 6.4 per thousand. In syphilis, Lange found normal, or with increased permeability, a somewhat decreased amount of chlorides, generally about 7.2 per thousand, but not below 7 per thousand, which he considers is already a decrease if the method used is a reliable one. On the other hand, Depisch, Richter and Quittner found an increase of chlorides in syphilis in almost every case. As to calcium,

Depisch and Richter-Quittner find a fairly constant value of 2 to 3 mg. per cent., i.e., less than in the plasma, which contains calcium-albumin compounds that are not diffusible. With a low or absent calcium content the writers found invariably violent headaches which were arrested by the oral administration of calcium. (This increases the calcium content of the spinal fluid.) Increased calcium content was also usually, though not invariably, accompanied by violent headache. The calcium could only be decreased by the withdrawal of spinal fluid.

Albumin is found in normal spinal fluid to the amount of about 0.2 to 0.25 per thousand. (Lange states 0.18 to 0.19, and he regards 0.2 per thousand as already pathological.) Lecithin is said to occur also to the amount of about 0.22 per thousand. Reducing substances, composed almost exclusively of dextrose, are found, according to Borberg, in quantities of 0.5 to 0.75 per thousand; Kahler gives 0.6 to 0.8 per thousand, Lange 0.5 to 0.56 per thousand, Depisch and Richter-Quittner 0.7 to 0.8 per thousand, Wittgenstein 0.45 to 0.6 per thousand. The value is lower than in the blood, probably because a part of the blood sugar is colloiddally combined ("kolloidal gebunden") — ["sucre virtuel"]. It is of theoretical interest that the sugar content of the spinal fluid shows an increase in slight degrees of disturbed permeability. Lange regards the combination of decrease of the chlorides and increase of sugar as the expression of the first stage of an inflammatory change in the meninges, which often appears to accompany general infections and intoxications, and therefore of little value in practice. (Cf. p. 185.) The sugar content is generally raised in epidemic encephalitis, which is of some importance in differential diagnosis. An increase of sugar in the fluid is also found in tumour of the brain, in epilepsy, apoplexy and encephalomalacia, and frequently in hypertonia. Decrease of sugar occurs in severe meningitic disease accompanied by heightened

permeability, i.e., in tuberculous and every other infectious meningitis. This was first pointed out by Mestrezat; the amounts are 0.3 to 0.2 per thousand, and sometimes only traces of sugar are present. The phenomenon has been referred by Sicard to a direct bacterial action, and by Mestrezat partly to absorption; Borberg thinks it an expression of an increased glycolytic function of the inflamed meninges, while Lange thinks the sugar is consumed by the cells of the fluid. The data in the literature as to syphilis are not unanimous; as a rule the sugar values are probably near the normal, although considerable decreases have been reported.¹

¹ It is not within the compass of the present book to give a minute description of the methods of investigation which can only be undertaken in the laboratory by expert chemists. But a few practical hints may be given which will facilitate the orientation of the scientific worker. Chlorides are generally estimated by Volhard's titration method, by which, when only extensive changes are to be indicated, the fluid, without having been previously treated, is submitted to the action of nitric acid; this method has been carried out by Hösslin for the estimation of sodium chloride in serum (Hofmeister's *Beitr.*, 8, 1906, p. 431). If minuter changes are required to be shown, organic substances must first be destroyed, with potassium permanganate according to Lange, for instance, or by the wet incineration method ("feuchte Veraschung") of Neumann or Korányi, Depisch and Richter-Quittner. For the estimation of sugar, Bang's micro-method (Borberg, Kahler), or that of Mandel and Steudel (Wittgenstein), and the potassium ferricyanide method of Hagedorn (Depisch and Richter-Quittner) have been employed. A comparatively simple, though less accurate, method which can also be employed by the non-chemist, is that recommended by Birkholz. One c.c. of spinal fluid is boiled with an equal volume of Haines' solution (copper sulphate, 2.0; distilled water and glycerine, $\bar{a}\bar{a}$ 15.0; 5 per cent. liquor potassæ, ad 150.0) and allowed to settle, when the colour should be compared before a frosted glass with blue test solutions of varying intensity. The test solutions are prepared by boiling solutions of glucose in graduated concentration (0.1, 0.125, 0.25, 0.5, 0.75 and 1.0 per thousand) with the reagent; 1 c.c. of each solution is boiled with 1 c.c. of the reagent. In a dark place and in closed tubes these solutions will keep for a fortnight, but it is advisable to renew them every week. For the estimation of calcium, Depisch and Richter-Quittner employed a method introduced by Jansen (*Zeitschr. f. phys. Chem.*, 101, 1918, p. 176). An apparatus for the estimation of urea, which substance readily passes the meninges, and in greatly increased amount sometimes indicates the approach of coma, has been given by Schumm (*Zeitschr. f. phys. Chem.*, 96, p. 335).

CHAPTER VIII.

QUANTITATIVE INVESTIGATION OF CELLS BY
THE COUNTING-CHAMBER METHOD.

THE counting of cells contained in the spinal fluid is now as a rule carried out by the counting-chamber method. I agree with the opinion of most writers that it is greatly superior to the French method, and that the parallel examination by both methods recommended by some writers is unnecessary. According to my experience the results obtained with the counting-chamber method are sufficiently reliable, provided the details of technique are carefully observed.

As the cells in different portions of the spinal fluid are not perfectly uniform in number—sometimes, indeed, considerable differences exist—it is best always to employ the same portion of fluid for counting purposes. I take as a rule the first portion by allowing the first ten drops of fluid to flow into a watch-glass. (Should the needle not be quite clear inside, I allow a few drops to run out first before collecting the fluid.) It is still more accurate to apply a pipette containing a sufficient quantity of the staining solution directly into the end of the cannula, and to draw the spinal fluid into the pipette as it flows out. (In given cases it may be desirable, as mentioned in Chapter I, to count the cells in the fluid from a higher region, e.g., in the last portion withdrawn. This procedure is to be recommended in focal affections of the cord. Weigeldt considers it essential that a considerable quantity of fluid should be removed with the patient in a sitting posture, and that at least the first and last portions be examined separately.) The further preparation

for counting should now be undertaken immediately. This rule, which was already advanced by Rehm, is unfortunately not always observed. Inaccurate results may sometimes be obtained when the fluid has been left standing some time, owing to sedimentation of cells, or to damage done to the cells by the fluid. As a few minutes must be waited before the pressure is measured, no time is lost in thus making the preparation for counting.

The accuracy of the pressure measurement is no doubt impaired a little by the removal of about $\frac{1}{2}$ c.c. of fluid for the investigation of the cells; but in cases in which the other changes in the fluid are of more importance than the determination of pressure, this is not of any serious consequence. In those cases where the determination of pressure is of special importance, or in which the removal of even small amounts of fluid without controlling the pressure is not indicated, e.g., in suspected tumours of the brain, I do not take the first portion of fluid, but measure the pressure first, and afterwards collect the fluid for the cell-count.

The preparation necessary for cell-counting consists in filling one of the Thoma-Zeiss mixing-pipettes for the enumeration of colourless blood-corpuscles, i.e., a mixing-pipette graduated 1 : 10 (which must, of course, be dry—after use, I wash it with methyl alcohol; and from time to time solution of potash should be passed through it), with the staining solution, previously filtered into a watch-glass, and the spinal fluid. The mixing is performed in reverse proportions to those customary for counting the colourless corpuscles in the blood, viz., the pipette is filled with staining solution as far as line 1, and fluid is drawn up to line 11, while for counting the colourless blood-corpuscles the blood is drawn up to line 1 (or 0.5) and the stain to line 11. The staining solution must therefore be considerably more concentrated in regard to stain and to acetic acid than is the case when count-

ing the colourless blood-corpuscles. Long ago I recommended the addition of a stronger solution of acetic acid (10 per cent.) than was otherwise customary, and recently I have become convinced of the excellent results achieved by the addition of a 50 per cent. solution of acetic acid, as recommended by Jacobsthal. The solution is as follows :—

Concentrated alcoholic solution of methyl violet	...	7.5 c.c.
Glacial acetic acid		25.0 c.c.
Distilled water	 ad	50.0 c.c.

If the same staining solution has been used for some time certain corrections are sometimes necessary, which presents no difficulty after a little practice. That the cell-staining varies in intensity in different specimens of spinal fluid by employment of the same staining solution, is due to the varying staining qualities of the cells—probably due to variations in their physical constitution. This is a cause of special difficulty in preparing well-stained cover-glass films.

After staining solution and spinal fluid have been drawn into the pipette, they are well mixed by carefully shaking the pipette for about two minutes, with the middle finger covering the pointed end, and the thumb at the other end, holding the free end of the rubber tube between the last two fingers. The mixing-pipette should be shaken horizontally, not vertically, and care should be taken that the glass bead within the bulb be heard to rattle while shaking, and that no air bubbles be allowed to enter the bulb. After having filled the pipette in this manner it may be placed aside, and further operations connected with the puncture can be proceeded with. The staining solution should act on the cells for at least fifteen minutes. If several punctures are undertaken, the pipettes must, of course, be labelled. Before filling the counting-chamber—which may be done on the following day, in which case it is necessary to cork the wide end of the pipette—the mixing-pipette must be again thoroughly shaken.

Instead of mixing in the pipette, Kafka recommends the following procedure : Into the cannula is placed a capillary pipette. The latter is provided with a conical end, but a piece of rubber connecting-tube would answer the same purpose. From this capillary tube he allows ten drops of spinal fluid to run into a clean vessel which already contains an equal-sized drop of staining solution, and mixes thoroughly. I agree with Eskuchen that this modification is inexpedient. According to my trials, it does not give equally constant results as those obtained by mixing in the mixing-pipette. The satisfactory results from the latter method may be shown by comparing two portions of the same spinal fluid which have been taken up into two mixing-pipettes. There is also a greater possibility of error in Kafka's method, as Eskuchen observes, in counting the number of drops. The advantages claimed by Kafka for his method, viz., rapid fixing of the cells, possibility of enumerating them at any time, are equally attainable with the mixing-pipette ; and the advantage of economizing fluid (0.2 instead of 0.4) and the mixing-pipette, is not of any weight. In my opinion his modification possesses an advantage only in cases where the spinal fluid has to be sent away for the enumeration of cells, in which event it is essential that it be previously fixed by the staining solution and well mixed with it. But no benefit is derived from this procedure, and it would seem desirable that all who carry out lumbar punctures should also be in a position to carry out the enumeration.

For the enumeration of cells in the spinal fluid, a counting-chamber is filled in the manner customary in the enumeration of blood corpuscles. The counting-chamber generally used is that of Fuchs-Rosenthal, or more recently that of Glaubermann after the type of Bürker's counting-cell for blood. The forms of counting-chamber generally in use for blood are hardly suitable owing to the small volume which they contain. The

rulings of the Fuchs-Rosenthal cell provide for sixteen large squares bordered by three lines, and each large square is again subdivided into sixteen small ones. The side of each small square measures $\frac{1}{4}$ mm.; the superficial area of a large square is 1 sq. mm., and that of a small square $\frac{1}{16}$ sq. mm., and the area of the whole is 16 sq. mm. The cell is 0.2 mm. in depth, not 0.1 mm. as in the blood-enumerating cells, and the volume of the whole cell is thus $16 \times 0.2 = 3.2$ c.mm. In order to calculate the number of cells in 1 c.mm. of spinal fluid, after having counted all the cells in the whole of the chamber, which is invariably necessary unless there is a

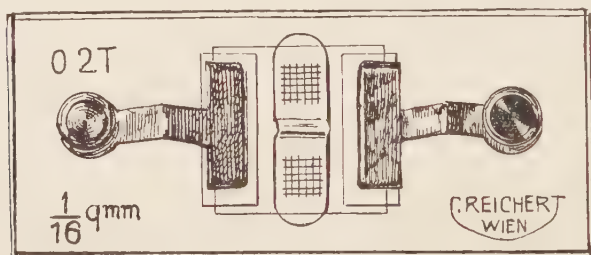


FIG. 5.

great increase of cells present, the number of cells found must therefore be divided by 3.2, and afterwards, as the fluid has been diluted as 9 : 10 (not as 10 : 11, as is often stated¹), multiplied by 10 and divided by 9; altogether, therefore, multiplied by $\frac{10}{3.2 \times 9}$, or divided by $\frac{28.8}{10} = 2.88$, or, though somewhat inaccurately, divided by 3.

The Glaubermann cell (see fig. 5) differs from that just described, firstly, in possessing, as in Bürker's hæmacytometer, two squares of rulings separated by a groove, and

¹ The proportions of the mixture are often distorted, as I admit after correspondence on the subject with Weigeldt, owing to diffusion, and if not performed accurately, owing to mixing in the (not always) capillary part of the pipette, in which case the ratio is about as 9.5 : 10.5.

secondly, that the depth of the chamber is obtained by the two ledges at the side for the cover-glass to rest on, being kept in position by two clips. This provides for a depth of 0.2 mm. above the level of the ruled lines.¹ This counting-chamber is easier to fill than others, being filled by placing a drop of the spinal fluid mixed with staining solution at the upper and lower edges of the cover-glass and allowing it to flow in, the cover-glass being already in position on the slide. Each of the two squares of

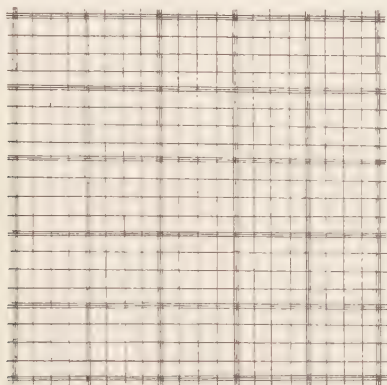


FIG. 6.

rulings is, moreover, larger than is the case with that of Fuchs-Rosenthal. Instead of 16 large squares it has 25, each of which has an area of 1 sq. mm. (see fig. 6). The volume above the rulings in the Glaubermann cell is, therefore, $25 \times 0.2 = 5$ c.mm. And the number of cells in 1 c.mm. of spinal fluid is found by dividing the total number of cells in both squares of rulings by 9, or the number in one system divided by 9 and multiplied by 2. (Owing to the dilution the volume of 5 c.mm. for one

¹ It is advisable to bend the metal clips a little so as to bring them further apart, otherwise there is not sufficient room for the objective.

system of squares, or of 10 c.mm. for both systems, must be multiplied by 9 and divided by 10.)

Before filling the counting-chamber one should not forget to blow out the two drops of undiluted spinal fluid contained within the capillary portion of the mixing-pipette. In order to fill the Fuchs-Rosenthal counting-chamber, a sufficiently large drop is allowed to fall on the ruled lines—the surrounding groove must be partly filled when applying the cover-glass—and the cover-slip is laid on the surface, preferably by sliding it from the side, avoiding the enclosure of air-bubbles, and the presence of Newton's rings verified. The ordinary thin cover-glasses may be used without disadvantage ($\frac{3}{4} \times \frac{3}{4}$ in., or $1 \times \frac{5}{8}$ in.). Owing to the greater depth of the counting-chamber, which necessitates a closer focusing of the objective than is the case for blood-counts, the Zeiss cover-glasses usually employed for this purpose are too thick when high powers are used. Having filled the counting-chamber, one must wait a few minutes to allow the cells to settle. With the Fuchs-Rosenthal cell the enumeration can be carried out some considerable time after having filled it, because the contents do not dry so quickly. If need be, however, the edges must be sealed with paraffin wax.

The best objective to use is the Zeiss DD, or the Reichert 6 or 7a; with eyepieces No. 2 or 4. Care should be taken when focusing that the iris diaphragm is shut down as much as possible, as otherwise the cover-glass is liable to be depressed; the illumination may then be increased, though as a rule the light must not be too strong. An attachable mechanical stage is a valuable help in counting; but with a little practice it is possible to move the slide perfectly well by hand, provided it is held down on one side by a stage clip. The cell-count is performed in the same way as in a blood-count, by counting the cells in all the longitudinal or the horizontal rows one after the other, while observing the rule throughout that cells lying on the lines are always to be counted

on the left and upper sides of the squares. The beginner should make a note of the number of cells found in each square, or at least in each row, so that he can control the written total at any point, or let it be controlled.

The counting-chamber method suffices the practical requirements perfectly, as I have remarked. The objection sometimes advanced against it that only a very small amount of fluid, i.e., only a very minute fraction of the whole, is examined, is of a theoretical nature; for the limits of error attaching to the method are very small. According to Walter's investigations, they amount to 100 per cent. for one cell to the cubic millimetre, 20 to 40 per cent. up to 5 cells to the cubic millimetre, from 15 to 30 cells 10 to 20 per cent., and for numbers above 30 cells to the cubic millimetre, 5 to 15 per cent. Weigeldt finds the error for 1 to 10 cells to average 21.5 per cent., from 10 to 25 cells 11.7 per cent., for 25 to 50 cells 7.1 per cent., from 50 to 200 cells 4.3 per cent., and for more than 200 cells an average error of 3.6 per cent. It will not do, therefore, to assert in general terms, as, e.g., Stern asserts, that differences up to five cells per cubic millimetre cannot be taken into account; for just in the cases with small numbers of cells on the border between the normal and the pathological, in which such minute differences are solely of any importance, the number of five cells goes much beyond the limits of error here given which prevail under careful technique of investigation.

CHAPTER IX.

DIFFERENTIATION BETWEEN CELLS IN THE COUNTING-CHAMBER. SIGNIFICANCE OF ADMIXTURE OF BLOOD FOR THE CELL-COUNT. CLINICAL DATA ON THE NUMBER OF CELLS IN THE SPINAL FLUID. MODIFICATIONS OF THE COUNTING-CHAMBER METHOD. ENUMERATION OF CELLS ACCORDING TO THE FRENCH METHOD.

THE staining in the counting-chamber is as a rule sufficient to enable the mononuclear cells of the spinal fluid to be distinguished from the polynuclear leucocytes, with their variously shaped nuclei. (The combined staining with eosin and methylene-blue, modified according to Dunzelt's method for the blood, has not given very satisfactory results in my hands; nor Birkholz's method of staining in the counting-chamber with methyl-green and pyronin.) An exact differentiation between the various kinds of mononuclear cells is not possible in the counting-chamber, although the typical small lymphocytes of the spinal fluid, with their strongly stained nucleus and their narrow, faintly stained protoplasmic margin, which is often only seen with difficulty, may as a rule be easily identified; and with a copious addition of acetic acid, nuclear fragmentation may be recognized in the medium-sized and the larger lymphocytes.

The peculiar occurrence may be noted, only seen in the counting-chamber, but not in cover-glass preparations, of certain faintly contoured, clear, unstaining or quite palely stained, circular discs, which lie at the periphery of some of the cells, often exceeding them in

size. These cells are to be found close to larger mononuclear cells, but also close to the typical small lymphocytes¹; usually there is only one disc to a cell, but occasionally two, and very seldom several discs to a cell may be seen. In normal spinal fluid they are rarely found; according to Rotstadt they are specially frequent in paralysis, but they also occur, sometimes in large numbers, in infectious meningitis.

The differentiation between the small lymphocytes and any red blood-corpuscles which may happen to be present, does not as a rule cause any difficulty, for with sufficient addition of acetic acid the red corpuscles are either completely destroyed, or so far decolorized as to be but very faintly yellow. Should the decolorization be insufficient, however, the inexperienced worker might mistake them for lymphocytes. (The beginner should, therefore, exercise the greatest caution; also sediment and stained dust-particles are, with insufficient experience, a possible source of error.)

The presence of a small number of red blood-corpuscles—up to three or four in the small squares of the counting-chamber—is not of any practical consequence to the result of the cell-count. Greater admixtures of blood interfere with the accuracy of the results, which in the case of border findings can be of considerable importance. A cautious evaluation of the counting results is possible by the use of certain measures of control, provided the admixture of blood is not too great. Such a control consists in making a separate count of the red blood-corpuscles contained as an admixture in the spinal fluid (count B), which cannot be undertaken, however, with the mixture of spinal fluid and staining solution, owing to the acetic acid present. The spinal fluid should

¹ Rotstadt has given a detailed description of these bodies—he calls them bubbles (“Bläschen”); Plaut, Rehm and Schottmüller also figure them in their “Leitfaden,” describing them as a peculiar finding in a tumour.

be mixed under similar conditions as before (in count A), or, in the case of a considerable admixture of blood, in the proportion customary in counting the colourless blood-corpuscles, viz., spinal fluid up to line 1, diluting liquid up to line 11; but instead of mixing with the staining solution, physiological salt solution is employed for the mixture. As the proportion of red to colourless corpuscles in the blood is about as 600:1, the number of colourless corpuscles which have entered the withdrawn spinal fluid accidentally as part of an artificial hæmorrhage is the 600th part of the number of red blood-corpuscles which it contains. It is therefore necessary to divide the number of red blood-corpuscles found in count B to the cubic millimetre of spinal fluid by 600, and to subtract the quotient from the number of the colourless cells to the c.mm. found in the chief count. If, for example, the findings in count A of the spinal fluid with blood admixture show seventeen colourless cells to the c.mm., and in count B the presence of 3,000 red blood-corpuscles to the c.mm. of spinal fluid, then the number of colourless cells to the c.mm. of fluid is not seventeen, but $17 - \frac{3000}{600} = 12$ cells.

A second control is offered by a differential count of the polynuclear cells and the lymphocytes in the counting-chamber, or if the staining here is insufficient, in a film preparation. It is based on the fact that the polynuclear leucocytes of the blood represent approximately 75 per cent. of the total of colourless blood-cells, and that therefore of the cells which have entered the spinal fluid through injury to a vessel about three-quarters are polynuclear cells and only a quarter can be mononuclear cell-elements. Accordingly, if from the total number of colourless cells to the c.mm. of spinal fluid all the polynuclear cells are subtracted, and further still a quarter of their number (corresponding to the percentage of mononuclear cells in the blood); if then there remains a marked pathological cell-value—not too near to the

border—it will then be possible to say that a lymphocytosis of the spinal fluid is present. The negative conclusion of the absence of an increase of cells, assuming that after subtraction of all the polynuclear cells and their corresponding number of mononuclear cells no pathological value should remain, is only to be drawn with reserve, for polynucleoses of the spinal fluid sometimes occur, though only in rare cases, so that the polynuclear cells found have not necessarily originated from the blood admixture.

Sometimes it is possible to infer the presence of a pathological cell-increase from the number of colourless cells counted, by taking consideration of the appearance of the blood-tinged spinal fluid. If, for example, 100 colourless cells have been found to the c.mm., the fluid ought to contain, if all these cells are due to blood admixture, $100 \times 600 = 60,000$ red blood-corpuscles to the c.mm., that is, the blood admixture ought to amount to more than 1 per cent., as blood contains about 5,000,000 erythrocytes to the c.mm. The appearance of the fluid, after well mixing of course, will sometimes show that such a large admixture cannot be present, and that the cells found cannot all owe their origin to the blood.

Apart from the artificial admixtures of blood just discussed, polynuclear leucocytes are normally absent from the spinal fluid; and in pathological cases (infectious meningitis excepted) only a low percentage of the total number of cells are polynuclear. But lymphocytes form in small number a part of the normal spinal fluid, and in cases of pathological cell-increase they represent as a rule the main bulk of this increase. The views as to what number of cells is to be considered normal are not unanimous. While some workers regard as pathological any increase over 2 to 3 cells to the cubic millimetre, others only speak of indubitable pathological findings beyond 15 cells to the cubic millimetre. I agree with other writers in regarding up to 5 cells to the cubic

millimetre as normal, 6 to 9 cells per cubic millimetre as conditionally pathological, i.e., abnormal as a rule, particularly when other slight deviations from the normal are present,¹ while I know of cases in which I have not felt justified on the ground of such findings in assuming a recovery from syphilitic infection, or any other disease. Cell-values from 10 onwards to the cubic millimetre I regard as pathological.

Increase of cells in the spinal fluid (Fischer rightly uses the non-committal term "pleocytosis" instead of the frequently used expression "lymphocytosis") is found—apart from acute meningeal diseases of varied ætiology, in which the findings sometimes go into the thousands of cells to the cubic millimetre—occasionally in various general diseases, and also in a certain, not generally high, percentage in all organic affections of the central nervous system; in non-syphilitic diseases, however, as a rule only in small amount. Syphilis of the central nervous system is specially characterized by the frequent occurrence of increase of cells,² which in most cases reaches a high degree; but syphilitic infection, especially in the secondary stage, is often associated with a high degree of pleocytosis without any manifest disease of the central nervous system.³

A few modifications of the counting-chamber method

¹ It is probably also right, as Gennerich says, that these border figures "may be regarded as idiopathic as soon as they appear to be retrogressive."

² Thus, in paralysis pleocytosis is found in about 98 per cent., in tabes in about 80 per cent. of cases. As a probable mean of the number of cells, I found in tabes 35 cells, in paralysis 55 cells to the cubic millimetre; in more than half my observations in cases of paralysis the number was 50 to 60 per cubic millimetre. Recent cases of genuine syphilitic nerve lesions are often characterized by an exceptionally large increase of cells.

³ The data in the literature as to pleocytosis in the early stage of syphilis vary between 23 and 80 per cent. As most of the results are based on a single investigation in each case, and the changes in this stage are doubtless transitory, it may be assumed that it very frequently occurs.

have not become generally adopted. With the idea of lessening the error that might attach to the examination of too small a quantity of fluid, Jones has suggested, in cases with a small number of cells, first to centrifuge the spinal fluid and then—after careful mixture with the stain, of course—to count the cells according to the directions given for the counting-chamber method. Laignel-Lavastine and Szécsi recommend this procedure to be followed regularly for the sake of the uniform results thus obtained. The calculation of the findings is simple: The number of cells to the cubic millimetre is found by taking the number of cells counted in the chamber and reduced to 1 c.mm., then multiplying it by the volume of the sediment expressed in c.c., and dividing it by the volume, also in cubic centimetres, of the fluid previous to centrifugation. Thus, supposing 3 c.c. to have been centrifuged, and the fluid removed all but a remainder of 0.3 c.c., then the number of cells found per cubic millimetre is to be divided by 10. The narrow end of the centrifuge tubes must, of course, be suitably graduated. A disadvantage attached to this method of investigation, which takes up much time when there is a large increase of cells, lies in the necessity of remixing the sediment, by which it is scarcely possible to get an even distribution.

Geissler also aims at increasing the volume of fluid in which the cells are counted, and he combines it at the same time with a better staining after the "French method." He uses a slide with a series of parallel rulings, and by means of a graduated pipette he places a drop of fluid containing exactly 40 c.mm. on the rulings. Then, after having stained, he counts all the cells which this drop contains, following the rulings. Compared with the counting of 3, 5, or 10 c.mm. of fluid in the Fuchs-Rosenthal, or the Glaubermann counting-chamber, this method necessitates a quite considerable expenditure of time. Geissler finds six to nine cells as the normal

number to the cubic millimetre—total number found divided by forty.

Walter, not possessing a counting-chamber, devised the following procedure: Into a mixing pipette for colourless corpuscles, spinal fluid as fresh as possible is drawn about as far as line 1. The end is then dried and so much fluid sucked out with a bit of linen until the upper level is exactly on a graduated line. The pipette is then placed vertically on a well-cleaned glass slide, and a certain amount, say five graduations, is released. In the mixing-pipette which Walter employed, one graduation was equal to 2.31 c.mm., or 11.6 c.mm. of fluid was employed for counting. When the pleocytosis is very great he takes a smaller volume. With both the foregoing methods of cell-counting a mechanical stage is essential.

While the principle of counting in the chamber is based on enumerating all the cells in a known, relatively small, volume of spinal fluid, in the so-called "French method" only the number of cells contained in a sample taken of a larger volume of spinal fluid are counted, from which the mean value is then calculated.

The method, which owes its origin to French writers, whose services in connection with the diagnostic value of lumbar puncture have been mentioned, is for us mainly of historical interest; but the method still has many adherents,¹ especially in France. According to the directions given by Widal, Sicard and Ravaut, 3 to 5 c.c. of spinal fluid is collected in a sterile centrifuge tube with a drawn-out end, and centrifuged rapidly for ten minutes. The fluid is then poured off and, holding the tube all the time inverted, a capillary tube is introduced vertically and the sediment taken up without suction. The sediment is then distributed over three or four glass slides, and the drops should not exceed 2 to 3 sq. mm. in

¹ Szécsi, for instance, also considers that "counting the cells in a film preparation suffices for ordinary clinical purposes."

area. These are then dried, fixed and stained, and cell-counts made in a number of fields of view of the immersion-lens, and the mean value determined. According to Sicard, in normal cases this value must not exceed three or four cells in a field with 400 to 500 magnification. Ravaut distinguishes between different degrees of the findings: 0 to 3 cells in the field of view = zero ("réaction nulle"); 4 to 6 cells = discrete ("réaction discrète"); 7 to 20 cells = average ("réaction moyenne"); and from 20 to 150 cells in the field of vision = high ("réaction grosse").

The somewhat inexact directions of the French writers have induced the numerous scrutinizers of their method to suggest a few modifications. Nissl suggested employing the centrifuge-tubes and pipettes not more than once; he also blows the sediment out of the capillary pipette and takes it into the pipette again, in order to secure a better distribution of the cells. E. Meyer advises scouring the drawn-out end of the centrifuge tube with the end of the pipette; Merzbacher distributes the drop by means of a capillary tube blown out at the end to a little ball. More exact directions for the rapidity of centrifuging were first given by Nissl. He centrifuges for three-quarters of an hour at 1,700 to 1,800 revolutions.¹ The volume of spinal fluid to be centrifuged, which was somewhat vaguely stated by the French writers, has also been given more precisely with a view to obtaining more equable results. Fischer thus advises to centrifugate invariably 3 c.c. of spinal fluid. Another improvement due to Fischer consists in always distributing the sediment over a surface of equal area, viz., an area of 2 sq. mm. (two cover-glasses, each 1 mm. square). Fischer finds by this method a border-value of two cells

¹ The object of these directions lies in stating the conditions which secure the complete deposit of all the cells in the spinal fluid—though the possibility of this is questionable—or which give at least comparable results.

in the field of vision of the immersion. Liebscher recommends the expression of the cell-count by a fraction with the formula: $\frac{\text{min.} - \text{max.}}{N}$, in which "min." is the smallest, and "max." the largest number of cells counted in a field of view, and N the arithmetical mean of all the counts.

A very approximate, yet simple, and roughly serviceable estimation of the number of cells is to be obtained by Nissl's "drop method." On a glass slide he rules in several places small meshes of horizontal and transverse lines, and distributes the whole sediment over these rulings in the form of three to five small drops of 2 to 3 c.mm. each, which he allows to dry, without spreading them. After having been fixed and stained, the drops are examined under a magnification which includes a whole droplet in the field of view, and on the strength of this total picture he establishes the presence of low ("luetic") or high ("paralytic") increase of cells.

Another modification of the French method is the following: Alter employs small glass frames holding exactly 1 c.c., which are fastened to a slide with paraffin wax. The frames are then filled with $\frac{1}{2}$ c.c. of spinal fluid and $\frac{1}{2}$ c.c. of 10 per cent. formalin, and set aside for four hours under a bell jar at room temperature, after which the fluid is syphoned off with cotton wool. The slides are then dried in the inspissator at a temperature of 37° C., then placed for a moment in an inspissator at 58° C., after which the glass frame can be lifted away; any remaining wax is removed with xylol, and it is then stained. Alter finds normally 0.5 to 0.7 lymphocytes in the field of view with a magnifying power of 385 $\times$.

CHAPTER X.

QUALITATIVE INVESTIGATION OF CELLS.

WHILE the numerical determination of cells in the spinal fluid is of great practical importance; the results which the qualitative investigation—the differentiation between the various kinds of cells in the fluid—is able to supply, have for the most part hitherto been of less importance from a practical point of view. To the few established, older facts, more recent works have contributed gratifying additions towards differential diagnosis; but much in this branch of the subject, while raising some interesting problems, appears to be still insufficiently founded.

The most frequent variety of cell in the spinal fluid, which is also found in normal conditions, is the spinal fluid lymphocyte, often simply called the spinal fluid cell (“Liquorzelle”). The question of its origin has been briskly controverted. Just as it is still an unsettled question whether the lymphocytes of tissues originate in the blood (Neisser, Marschalkó), or in the tissues (Unna, A. Pappenheim, Naegeli), so the same differences of opinion have prevailed as to the origin of the spinal fluid lymphocytes. While, for instance, Nissl refers to them as “blood-lymphocytes,” Fischer upheld the view, from collateral researches of the fluid and the meninges, that they originated in the tissues; and Szécsi attempted to prove from the staining qualities of the spinal fluid cells that the bulk of them did not have their origin in the blood. It is not improbable that Klien’s intermediary point of view is the right one. His view is that all the various sources of origin that come into consideration for

mononuclear exudate-cells play a part in the origin of the lymphocytes of the spinal fluid, viz., from indifferent wander cells (the lymphocytes of tissues), from the resting wander-cells of connective tissue (Maximow), particularly the adventitia cells of Marchand belonging thereto; on the other hand also migration from the blood- and lymph-vessels, and finally transformation from the epithelial-cells of serous membranes.¹ Nevertheless the larger proportion no doubt originate in the tissues.

The qualitative investigation of spinal fluid cells discloses numerous differences in their form, and especially in their size. Klien classifies them according to size into three gradatedly connected groups, the dimensions being taken from cells stained by the supravital method, viz., small forms up to $9.6\ \mu$ in diameter (about the size of red blood-corpuscles), of which the smallest are less than $5\ \mu$ in diameter, medium-sized forms from 9.6 to $11.2\ \mu$, and large forms with a still larger diameter (up to about $13\ \mu$). The lymphocytes are distinguished by their plump, coarse-threaded nucleus, which is either roundish or flattened, crenulated, notched, lobed or even (according to Klien) sometimes fragmented. Cells with this form of nucleus are distinguishable from polynuclear leucocytes by the structure of the protoplasm, which is not neutrophile granulated, but is basophile; and by the presence of a nucleolus which is generally in the largest part of the nucleus. In the small lymphocytes the nucleus almost fills up the whole cell; in somewhat larger cells a narrow margin of protoplasm is distinctly visible; in medium-sized cells there is a considerable, invariably ungranulated protoplasmic portion, but the nucleus, which lies eccentrically in larger cells, always takes up the largest part

¹The wander-cells found by Kolmer (*Anatom. Anz.*, 54, 1921, 15) in all the vertebrate classes on the surface of the choroid plexus come scarcely into consideration as a source of origin of the spinal fluid cells, for the normal ventricular fluid is generally free from cells, and in pathological conditions it only very rarely contains an abundance of cells (Weigeldt).

of the cell; nuclear and protoplasmic increases of size do not always run parallel together. The common characteristics of all lymphocytes are the coarse nuclear structure, the relative largeness of the cell-nucleus, and the absence of granulation in the protoplasm.

Besides these cell elements Rehm has described tailed lymphocytes which only occur in pathological specimens of spinal fluid. These are characterized by their protoplasm being drawn out like a tail, into which an extension of the irregularly-shaped nucleus sometimes projects. Szécsi and Rotstadt, and recently Weigeldt, consider these tailed forms to be artifacts, brought about partly by alcohol-hardening and partly by inappropriate spreading of the sediment: while Klien, whose view I share, thinks that such cells occur in small numbers in pathological conditions, but that the particular frequency in which Rehm observed them is to be traced to the circumstance that in the Alzheimer technique which he employed, the centrifugation deforms the alcohol-hardened cells, and the more so the sooner the centrifugation takes place, for when hardened for a longer time the cells are better able to resist the deforming tendency.¹

Normal spinal fluid contains only small and medium-sized lymphocytes, never those with a greatly enlarged nucleus. According to Klien a comparatively greater number of larger forms occur in acute inflammations, while in very chronic cases the cells are nearly all of the small variety, with very few large ones. Rounded, or only slightly notched, nuclei are characteristic, moreover, in acute disorders. The tailed forms are found chiefly

¹The researches of Bergel (*Berl. klin. Woch.*, 1919, No. 39 and elsewhere) on a fat-splitting ferment contained in the lymphocytes are of interest in the question as to the occurrence of tailed lymphocytes. When oils are injected into the thoracic and peritoneal cavities of guinea-pigs and rabbits, Bergel found after a time "pronounced pseudopodia-like extensions in the smaller and medium-sized lymphocytes." Bergel credits the lymphocytes, including the small ones, with amœboid movements and phagocytic qualities.

in cases of fresh lesions. Klien's researches thus lead to the following conclusions : In acute disorders there occur chiefly large cells with a rounded nucleus ; in chronic lesions small cells with a notched and fragmented nucleus predominate.¹

Klien also endeavours to draw conclusions as to the extent of inflammatory lesions from the inter-relationship of the form of the cells to their number. Thus, a large increase of cells accompanied by a high percentage of lymphocytes with nuclear fragmentation, i.e., in a chronic disease, speaks in favour of a correspondingly large extension of the meningeal lesion ; while on the other hand, should there be found, notwithstanding little nuclear fragmentation, only a small percentage of lymphocytes to the cubic millimetre, this would indicate that the comparatively acute lesion is confined to a circumscribed region, so that a relatively small amount of pleocytosis has taken place in spite of an abundant expulsion of lymphocytes in the affected parts. Uncertainty still prevails as regards the large mononuclear cells with a large share of basophile, ungranulated protoplasm and an irregularly-shaped or lobed nucleus. Szécsi describes them as large monocytes and opines they are to be regarded partly as "monocytes" in the sense of A. Pappenheim (they correspond to Ehrlich's large mononuclear leucocytes and transitional blood-cells) ; and partly as having arisen from epithelial cells, without, however, being in a position to distinguish between these two modes of origin. Bergel reckons them among the lymphocyte group, and designates them as pathognomonic "of a stage of increased cell-activity."

Besides these cells Szécsi describes elements which are

² I have repeatedly observed a strong preponderance of large cells in cases of syphilo-meningitic changes in neuro-relapse. I saw a striking number of tailed cells in a case of herpes zoster. In two cases of uræmia with pressure-increase and slight increase of albumin I found very little increase of cells, but almost exclusively large ones, partly, perhaps, of an endothelial character.

only distinguishable from the small lymphocytes by the more delicate structure of their chromatin, and which he calls microlymphoidocytes; also certain rarely occurring larger cells of the same variety, occasionally with several nucleoli, the lymphoidocytes, from which the microlymphoidocytes are said to take their origin. Szécsi regards the circumstance that these cells were found in the spinal fluid, while not occurring in the blood of healthy persons or in the blood of such cases of disease in which he performed lumbar puncture, as proof of the histogenous origin not only of these cells but of the overwhelming majority of all the spinal fluid cells.

The polynuclear leucocytes, of whose derivation from the blood there is no doubt, which also agree in appearance with the polynuclear blood-corpuscles—though in the spinal fluid their neutrophile granules are often not easy to demonstrate—are known to chiefly characterize the spinal fluid in acute infectious meningitic diseases, and they possess a certain value in differentiating between these and tubercular meningitis. In rare cases a polynucleosis of the spinal fluid is the expression of a meningeal reaction to toxic, mechanical, or thermal stimuli. Polynuclear leucocytes also occur in small percentage in the spinal fluid in cases of chronic meningeal lesions, particularly in progressive paralysis.

True eosinophile polynuclear leucocytes, i.e., cells with typical eosinophile granules,¹ are very rarely found in the spinal fluid. Grund has observed them to occur in cysticercosis of the brain; Rotstadt saw eosinophilia

¹ The "peculiar eosinophilia" of the polynuclear leucocytes of the spinal fluid, pointed out by O. Fischer, I was able to trace to the fact that the spinal fluid cells have in general a greater affinity for stains than the blood cells. After the polynuclear leucocytes have been subject to the action of the spinal fluid for some considerable time, i.e., when they are less recently extravasated specimens, the stainability of their protoplasm decreases, which explains the difference between the more "eosinophile" cells and the weaker staining protoplasm of the polynuclear cells.

in a case of paralysis at a time of long-continued convulsions.

Most writers on the subject have never observed mast cells, i.e., basophile polynuclear leucocytes, in the spinal fluid. Klien found similar cells occasionally, however, which differ from the mast cells of the blood by their coarser and somewhat rounder granules, and particularly by their nuclear structure and the well-developed nucleoli.

In pathological spinal fluid, besides lymphocytes and polynuclear leucocytes, a third variety of cells are often found in not inconsiderable numbers, the plasma-cells. These were first shown by Milian to occur in tubercular meningitis, and by O. Fischer in paralysis. They are chiefly characterized by the strongly basophile nature of their protoplasm (granoplasm, spongioplasm), and, further, by the pronounced chromatin network of their often eccentrically placed nucleus. Other characters of the plasma-cells of tissues, e.g., radial arrangement of the chromatin, and perinuclear clearness of the protoplasm (brighter ring, free from spongioplasm, around the nucleus), are not regularly met with, and are usually not so pronounced as in the corresponding cells of tissues. On the other hand, the lymphocytes of the spinal fluid sometimes exhibit the same phenomena, which are to be seen in all degrees between these and typical plasma-cells. Here, again, however, it is an open question whether, according to Nissl, the plasma-cells of the spinal fluid develop in the blood from lymphocytes and then emigrate, or whether their origin (according to A. Pappenheim, Weidenreich, O. Fischer) is exclusively or mainly autochthonous in the tissues from histogenous lymphocytes.

Also the plasma-cells sometimes exhibit polymorphism and fragmentation of the nucleus, and occasionally tailed protoplasm. Plasma-cells are found in the spinal fluid in most pleocytoses of longer duration; and they are

specially abundant in many cases of nervous lesions in later syphilis, without showing any special differences in the various diseases of this group, whether paralytic, tabetic, or cerebro-spinal; but they are also found in early stages of syphilis without any manifest affection of the central organs; and in chronic or abating cases of meningitis they sometimes occur in not inconsiderable numbers. Klien holds the view that they represent functional states of the cells which may possibly stand in relationship to the mechanism of immunity, but that they are not degenerative forms as is sometimes thought from their phagocytic qualities. It is noteworthy that in the salvarsan treatment of syphilitic nervous lesions, the plasma-cells disappear quicker from the spinal fluid than the other cells, as Szécsi points out.

In addition to the cells described, there are still a few rarely occurring varieties of cells, which are found in small numbers, e.g., connective-tissue corpuscles ("fibroblasts"), fusiform cells often of great length (according to Klien up to $96\ \mu$), and with a delicately staining nucleus which often lies to the side of the cell instead of in it. Some of these cells are phagocytic, in others the cell consists of fine longitudinal fibres that sometimes appear to enmesh the nucleus. The fibroblasts are most often found in lues cerebri and progressive paralysis.

Other phagocytic cells of very varied appearance are summarized under the name of macrophages (it has already been mentioned that plasma-cells are occasionally phagocytic). A certain proportion of them are large, round, oval, or more polygonal cells, sometimes ten to fifteen times as large as the lymphocytes. They contain much protoplasm and a comparatively small nucleus which occupies only a quarter to a third of the cell and is usually situated at the margin. The nucleus appears homogeneous or with a delicate network. The cells usually contain all kinds of enclosures, such as: lymphocytes, polynuclear leucocytes, red blood-corpuscles, blood

pigment, fat-globules, bacteria, &c. In some of these cells vacuoles are developed, which sometimes unite to form a single vacuole which fills out the whole body of the cell; but sometimes they are separated by thin partitions, when they are known as lattice-cells ("Gitterzellen"). Nothing certain is known as to the origin of the macrophages; from lymphocytes and emigrated monocytes from the blood, from capillary endothelia, from the resting wander-cells of connective tissue, from adventitia cells, and particularly, it would seem, from the endothelial cells of the cerebral membranes, all come into consideration as the sources of their origin.¹ Brückner looks upon the lattice-cells as elements of a glial nature which have wandered into the spinal fluid, or as altered ependymal or plexus epithelium-cells. The macrophages and the lattice-cells are chiefly found in progressive paralysis as well as in infectious meningitis.

The exceptional occurrence of neutrophile myelocytes in leukaemia, according to Barker, may still be referred to. Schwarz, Mohr, and others have found, moreover, occasional instances of tumour elements in the spinal fluid recognizable by their size and appearance, as well as by their karyokinetic features; such fortunate accidental discoveries can be of great diagnostic importance.²

¹ In a case that came under my observation, after a local inflammatory reaction that had supervened upon a lumbar puncture performed some time previously with artificial admixture of blood, the spinal fluid was found to contain a large number of cells containing hæmosiderin (the hæmatomacrophages of Sabrazet and Muratet), which had been manifestly derived from polynuclear leucocytes.

² Fischer states that in histologically malign tumours which reach at least as far as the meninges, one can find as a rule, with formalin fixation *in vitro* (see note p. 97) and hæmatoxylin-eosin staining, small but characteristic tumour-cells, and this is of great value in practice, as in such cases an operation need not be contemplated and X-ray treatment should be tried. These cells may be recognized by (1) their usually somewhat larger cell-body, and often angular, sometimes markedly hexagonal, outline; and (2) a more vesiculiform, but often variously lobed, nucleus

The spinal fluid not infrequently contains numerous degenerative forms due to the injurious action of the fluid leading to disintegration of the cells.¹ The lymphocytes often exhibit changes; their nuclei become unstaining, and more and more homogeneous until finally small, homogeneous, round bodies, devoid of structure, result. A very narrow protoplasmic margin is then present, or it may be absent; and sometimes the cells contain fat-globules and vacuoles.

The macrophages also tend strongly to degenerative changes, which are expressed in a similar manner by the nucleus becoming homogeneous and the boundary between nucleus and protoplasm disappearing. The occurrence of altered erythrocytes after hæmorrhage in the subarachnoid space has been referred to in dealing with admixture of blood.

¹ I have studied this injurious action experimentally and have described in detail the degenerative forms produced in colourless blood-corpuscles by the spinal fluid. Weigeldt has recently confirmed these researches and especially the disintegration of the polynuclear cells.

CHAPTER XI.

QUALITATIVE INVESTIGATION OF CELLS :
METHODS.

THE technical difficulty attached to the staining of the spinal fluid cells is responsible for much of the contradiction and the vagueness in the research work on the subject. Leaving aside the sufficient divergence of opinion in interpreting the cells of the blood, the most experienced worker but rarely obtains such good results in staining the spinal fluid cells as is the case with blood preparations. This is partly due to the tendency of the spinal fluid cells to break down ; but chiefly to their totally different staining affinity from that of the blood cells. I was the first to point this out, and to refer the circumstance to the poorness of the cerebro-spinal fluid in albumin. The staining proportions customary for blood-preparations and the duration of staining must therefore be considerably modified. In spite of all efforts to this end, however, a reliable and effective staining method for the spinal fluid does not exist ; and this is due to the fact, as Kafka incidentally remarks, that for each specimen of spinal fluid a special staining-mixture ought really to be prepared.

The methods of staining the spinal fluid most generally in use are as follows : The "French method" of staining films on cover-glasses and slides, due to Widal, Sicard and Ravaut ; the Alzheimer method, according to which the spinal fluid cells are embedded and sections cut in the manner adopted for tissues ; an interesting "supravital" method owes its origin to Klien. The investigation of unstained cells in the hanging-drop

according to Frenkel-Heiden (*Neurol. Zentralbl.*, 1912, p. 1085), though of interest is not of much value.

For smear preparations a certain quantity of spinal fluid, about 3 c.c., is centrifuged in a tube with a narrow end (see fig. 7)¹. (If necessary one can make these from suitable glass tubes.)

After having poured off the fluid the tube is inverted on a piece of blotting-paper to drain, so as to thicken the sediment as much as possible. The sediment is then drawn into a capillary tube provided with a suitably long, thin end (see fig. 7), then expelled several times and taken up entirely into the capillary tube. The sediment is then blown out on to a number of cover-glasses or slides, and

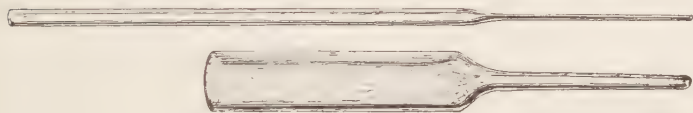


FIG. 7.

distributed by means of a glass rod or a capillary tube with the end fused to a small ball. Rotstadt rejects the spreading of the drop in this manner on the ground that it produces artifacts; he divides the drops into smaller droplets and allows them to dry. Lange makes on a glass slide a greasy line forming a circle about $\frac{1}{4}$ in. in diameter, and places the sediment in it. The volume of sediment should be so small that its surface has no convexity above the grease line, nor should it extend beyond it. After drying, which is best accomplished in the

¹ Fischer suggests adding formalin to the spinal fluid for the purpose of fixing the cells before centrifugation. A drop of formalin is added to each c.c. of the fluid immediately after removal, so that a 5 per cent. solution of formalin results. This procedure is an advantage if the fluid is not soon to be attended to; the addition of formalin is unnecessary otherwise. The damage done to the cells by centrifugation is, as I concede to Szécsi, not so great as Fischer thought.

inspissator at $37^{\circ}\text{C}.$, or on a wire gauze over a water bath at a temperature of 55° to $60^{\circ}\text{C}.$, the preparations are fixed and stained. Centrifuge tubes and capillary tubes should only be used once.

Among the methods of staining, the hæmatoxylin-eosin method of O. Fischer, after fixing on Ehrlich's copper plate at about $120^{\circ}\text{C}.$, is comparatively simple. Fischer recommends overstaining with hæmatoxylin, decolorization with weak hydrochloric acid, and counterstaining with eosin. I have obtained fairly constant results by fixing with methyl alcohol, then staining by the French method for three or four minutes in a $\frac{1}{4}$ per cent. alcoholic solution of eosin, well drying, and staining for three or four minutes in a Delafield hæmatoxylin solution diluted to one half. The hæmatoxylin-eosin staining has the disadvantage, however, that it is not a simultaneous but a successive staining; from the hæmatological standpoint it gives, as Szécsi rightly says, only very rough pictures, and is not able to show the plasma cells characteristically, owing to its lack of a suitable basic stain; notwithstanding the fact, however, that by its means Fischer discovered the plasma cells of the spinal fluid—although only some of the cells he regarded as such were undoubtedly plasma cells.

For the study of the finer structure and for the characteristic staining of the plasma cells, the employment of other staining methods is therefore necessary; though it must be said that they more often completely fail than the hæmatoxylin-eosin method. Szécsi has the merit of having transferred hæmatological methods to the staining of the spinal fluid, and a number of directions for staining are due to him:

(1) Methyl-green-pyronin staining: First fix for $\frac{1}{2}$ minute on Kowarski's copper plate at 120° - $130^{\circ}\text{C}.$; then fix for $\frac{1}{4}$ to $\frac{1}{2}$ minute in sublimate alcohol (after A. Pappenheim: hot concentrated solution of corrosive sublimate in physiologic saline solution; after cooling,

mixing in equal parts with alcohol) ; pour off the solution and pour distilled water over the cover-glass, followed by alcoholic solution of iodine, and finally alcohol ; dry and stain.

According to more recent directions, Szécsi fixes for ten minutes in an acetonic solution of benzoyl peroxide, and without drying places quickly in a mixture of acetone and xylol (3 : 2) and allows this to act for ten minutes, or longer ; then change over to methyl alcohol ; dry and stain. Stain for five minutes in the purchasable solution of A. Pappenheim's methyl-green-pyronin, or four minutes in a solution of equal parts of methyl-green and pyronin, with or without a previous treatment with a $\frac{1}{2}$ per cent. solution of Orange G. Decolorization can be easily effected after staining by slightly dipping in absolute alcohol.

(2) May-Grünwald-Giemsa staining: Dry in the inspissator and fix for one minute in May-Grünwald solution (this and Leishman solution, being solutions in methyl alcohol, have a fixing effect) ; then cover with ten to twelve drops of Giemsa solution, always freshly prepared (three drops of an older, or five drops of a new solution to 10 c.c. distilled water), and stain not longer than thirty seconds ; pour off the staining solution, and wash by moving the cover-glass back and forth in a fair-sized vessel of distilled water ; then dry in the air, and slightly decolorize with a drop of absolute alcohol.

According to more recent directions, Szécsi prescribes fixing only for ten to fifteen seconds in May-Grünwald solution, briefly rinsing with distilled water, and staining for ten to fifteen seconds with the above Giemsa solution in a staining glass. With this method of staining, the film can also be first partly fixed with benzoyl peroxide, when, after rinsing with methyl alcohol, only a diluted May-Grünwald solution (— distilled water) should be poured over the film, which is then to be stained in the Giemsa solution. Instead of the last mentioned,

A. Pappenheim's "panchrome" mixture may be employed.

Lange fixes for at least ten minutes with methyl alcohol, to which 1 per cent. of formalin may be added. He then stains for ten minutes with Giemsa solution, diluted at least as 1 : 100, frequently renewing the solution on the cover-glass. The preparations are more successful the weaker the staining solution and the longer they are stained in proportion.

(3) Leishman staining: After drying in the inspissator, fix in the Cornet cover-glass holder for forty seconds with Leishman mixture; then transfer to a staining-glass containing five drops of Leishman solution to 10 c.c. distilled water. Or cover the film drop by drop with fifteen drops of Leishman solution, and fix for forty to sixty seconds; then pour this solution into a glass with 1 c.c. of distilled water; quickly shake the mixture and stain in this for twenty seconds. If benzoyl peroxide has been employed for the preliminary fixing, the cover-glass must be conveyed direct from the methyl alcohol into the staining-glass containing diluted Leishman solution, and stained for fifteen to twenty seconds.

Schlüchterer, as in Alzheimer's method, precipitates the albumin prior to staining with a solution of 3 c.c. corrosive sublimate in 100 c.c. of a 1 per cent. solution of acetic acid. This solution is added drop by drop to 4 c.c. of spinal fluid in the narrow-ended centrifuge tube until the fluid becomes milky; it is then shaken. After centrifugation he does not imbed, as in the Alzheimer method, but makes smear preparations, which he places in an alcoholic solution of iodine (iodine added to 70 per cent. alcohol until it acquires a deep reddish-blue colour) for five to ten minutes with the film side upwards. It is then thoroughly washed in 70 per cent. alcohol until the iodine is dissolved out, and stained, e.g., with methyl-green-pyronin for ten minutes in the incubator: afterwards rinse and dry. Fairly good results are obtained

by this method. To obviate shrinkage of the cells, Bostroem in Nonne's ward employed instead of the acetic acid solution of corrosive sublimate, a concentrated solution of corrosive sublimate without glacial acetic acid (corrosive sublimate 7.5 : 0.5 per cent. solution of sodium chloride 100.0). Weigeldt distributes the sediment of the freshly centrifuged fluid in drops on cover-slips, and before drying allows them to float immediately, with the drop side down, on a hot alcoholic solution of corrosive sublimate for five to ten seconds, by which, it is true, a small part of the sediment falls away; then, without drying, he places the film in alcoholic solution of iodine and treats further as given above.

The procedure in Alzheimer's method is as follows: About 5 c.c. of cerebro-spinal fluid is allowed to flow directly from the cannula into 10 to 15 c.c. of 96 per cent. alcohol; this is then well shaken, the mouth of the tube closed with cotton wool, and centrifuged for half to three-quarters of an hour. A coagulum containing the cells is formed at the bottom of the tube. The supernatant fluid is now to be poured off and the coagulum hardened for an hour each with absolute alcohol, acetone and alcohol, and acetone. The coagulum is then extracted with a needle and embedded in celloidin, which is possible in one to two hours. Sections of about 15μ in thickness are cut with a microtome, and previous to staining, e.g., with methyl-green-pyronin, the celloidin is removed by means of methyl alcohol. In order to facilitate the removal of the sediment from the tube, Rotstadt recommends substituting aniline oil after pouring off the absolute alcohol. The sediment then often rises alone to the surface after ten to fifteen minutes; otherwise it is sufficient to release the sediment carefully from the side of the tube in one or two places, when the sediment will rise to the surface.

The embedding process is also applicable when other methods of fixing are adopted, e.g., with corrosive sub-

limate fixing and subsequent triacid staining; with Fleming's fixing and Altmann's staining; Zenker's fixing and Giemsa or toluidine-blue-chromotrope staining. When employing these methods of fixation, the sediment should be embedded in paraffin wax, and the sediment should be impregnated with xylol before removing it from the tube; the sections should be 3 to 5 μ in thickness. A disadvantage of Alzheimer's method is its complication, and the large amount of spinal fluid it necessitates.

Klien gives the following directions for his supravital method: The spinal fluid should be centrifuged as soon as possible after removal; when there is an abundance of cells present, the centrifugation should not be too vigorous, in order to avoid clumping together of the cells. After pouring off the fluid, the sediment must be treated with the staining solution as soon as possible. Klien employs a solution of methyl-green-pyronin equal in amount to the quantity of sediment, and allows it to act for one to several hours, during which period the mixture of sediment and staining solution is drawn several times into a capillary tube, and blown out again in order to secure an equal distribution of cells. A drop of the mixture is then placed on a slide, covered with a cover-glass and examined with the immersion lens.¹ Should it not be possible to examine immediately, the edges must be sealed with paraffin wax, otherwise the cells at the margin rapidly undergo a change of structure. Klien claims for his method the advantage that the cells remain well preserved, not contracting under the influence of the alcohol, or suffering from being dried, and that their dimensions can be measured in their natural size; while

¹ It is also possible to stain by mixing the spinal fluid with the staining solution and examining in the hanging drop. Birkholz (*Archiv. für Ohrenheilkunde*, 1921, vol. cviii) takes, when the fluid is clear, 1 drop of methyl-green-pyronin solution, and when cloudy, 6 drops, for staining 10 drops of spinal fluid.

the disadvantage attaching to the method is that the preparations are not permanent, for the optimum staining differentiation is often somewhat impaired after a few hours. If the margin of the preparation is well sealed the configuration of the cells is maintained unchanged for several days.

The chief varieties of cells have the following appearance under the various staining methods :—

The plasma cells can only be shown characteristically with methyl-green and pyronin. Their most striking feature is the vivid red staining of the cell-protoplasm ; but this is often interrupted by lighter unstaining parts, and the protoplasm sometimes appears laminated and lumpy. The nucleus, which is often situated eccentrically, is stained more or less dark green by the methyl-green ; it shows a pronounced chromatin network, which in cover-glass preparations is often more or less distinctly radial in arrangement. In supravital preparations the nucleus shows two to four, or even five, large, dark brown nucleoli. The perinuclear brightness is absent from supravital preparations, while in cover-glass preparations it may often be seen.

The lymphocytes exhibit a dark blue nucleus with the hæmatoxylin-eosin staining. The protoplasm, if scantily present in the form of a narrow margin, is usually pink ; in larger cells with a stronger development of protoplasm it is more bluish-violet. If the lymphocytes are altered the nucleus is unequally and less intensively stained, and the colour tends more towards red. With Leishman and May-Grünwald-Giemsa staining, the nucleus of the small lymphocytes is dark blue and coarsely structured ; and with longer staining by the May-Grünwald-Giemsa method, the nucleus may appear tinged dark brown, and in large cells a light blue. The protoplasm is stained light blue, with in some cells slightly rose-coloured parts shining through. The nucleus in lymphocytes stained with methyl-green-pyronin is intensely green, and is

sometimes so strongly stained as to obliterate all traces of structure. In the supravital method the nucleus is bluish to blue-violet, and shows a distinct chromatin structure. In this method of staining, which brings out with especial distinctness the notching and lobing of the nucleus, there is generally a fairly large, sharply outlined, metachromatically brown-stained nucleolus; sometimes two are present, rarely several. The protoplasm of the lymphocytes in this staining method varies from pale pink to deep pink and red.

The polynuclear leucocytes of the spinal fluid exhibit similar forms of nuclei to those we are acquainted with in the blood, dumb-bell-shaped, sausage-like forms, or a few small rounded nuclei. With hæmatoxylin staining the nuclei are dark blue; the protoplasm is pure pink, and with prolonged staining it becomes tinged bright red, sometimes unequally, without distinct granules. Degenerative forms show a pale bluish nucleus; in severely injured forms the nucleus is often scarcely distinguishable from the protoplasm. With Leishman and May-Grünwald-Giemsa staining the nuclei are stained light blue, the protoplasm pink; and with methyl-green and pyronin staining the nuclei are pale green, the protoplasm light brown.

CHAPTER XII.

INVESTIGATION OF PROTEINS.
 GLOBULIN REACTIONS: OF NONNE-APELT, OF
 ROSS-JONES. PÁNDY'S REACTION.

UNDER physiological conditions the spinal fluid contains, like the aqueous humour of the eye, a certain amount of albuminous substances, chiefly serum-albumin and globulin. In pathological conditions an increase of these protein substances takes place¹; on the one hand, in inflammatory changes of the meninges, i.e., in the various forms of infectious and symptomatic meningitis, in syphilitic disease of the central nervous system, and in syphilitic infection without manifest nervous derangements; to a smaller degree, and less frequently, in other organic diseases in which the central nervous system is involved, and occasionally as a transient symptom in general infections, &c.; on the other hand, in increased transudation due to stagnation of the spinal fluid.

The aim followed therefore in carrying out this investigation of the spinal fluid is to determine the presence of an increase above the normal of the total content in protein substances, and especially the globulins. The investigation of the globulins is based on the circumstance that they can be precipitated by the agency of certain neutral salts in a degree of saturation which leaves the

¹ Lange found subnormal values (0·15 per thousand and less) in secondary increase of spinal fluid, i.e., increase no longer due to inflammation, in chronic hydrocephalus, and in senile atrophy. He ascribes no practical importance to these findings.

albumins still in solution, e.g., by full saturation with magnesium sulphate (Hammarsten); or by half-saturation with ammonium sulphate (Hofmeister).

Such investigations already found mention in the first writings on the subject of the protein content of the spinal fluid, e.g., those of Widal, Sicard and Ravaut, Guillain and Parant, Monod—who all employed magnesium sulphate. Their outcome, however, was not of any great practical value, nor that of Nissl's researches with ammonium sulphate, or Cimbál's with zinc sulphate. Only since definite directions have been set up by Nonne, Apelt and Schumm, for the interpretation of their "globulin reaction,"¹ which is based on the same principle, have we been in possession of a very convenient and practical method of investigation, which has since become generally adopted. The reaction is rather coarse, however, as it is as a rule only positive from half per thousand upwards.

The reaction is performed by mixing together equal volumes of spinal fluid and a hot-saturated solution of ammonium sulphate, and after three minutes have elapsed comparing the mixture with a specimen of the spinal fluid not so treated. Originally, the writers took 2 c.c. each of spinal fluid and reagent, but smaller amounts will also suffice. The solution of ammonium sulphate requires a certain care in preparation. It is not advisable to heat it to the boiling point, as this causes ammonia to

¹ The expression "globulin reaction" is not quite correct, as albumoses and other protein substances are precipitated at the same time as the globulins. The former can occur in small amounts in the spinal fluid in pathological conditions, without any practical significance having been hitherto assigned to them. For this reason Cimbál has designated the proteins precipitated according to his method with zinc sulphate as Fraction I; and Nonne and Apelt have applied to those precipitated with ammonium sulphate the term Phase I. The albumins still remaining in the spinal fluid after salting out and filtering, which can still be separated by acidulation and boiling, received in the same way the designations Fraction II, or Phase II. The employment of the Phase II reaction has no practical value.

be given off and sulphuric acid to be liberated, thus giving rise to easier precipitable acid-albumins that can cause turbidity in specimens of normal spinal fluid also. For this reason every freshly prepared solution should be tested to ascertain if it is neutral, and the test should be repeated from time to time if the reagent is long in use. A very diluted solution of litmus tincture should be employed, as litmus paper does not absorb the thick solution. Should the solution of ammonium sulphate prove to be acid it is to be neutralized by the dropwise addition of solution of ammonia. For the preparation of a saturated solution about 85 gm. of chemically pure ammonium sulphate to 100 c.c. of distilled water will be required. The solution must be allowed to cool slowly, and white crystals must be deposited at the bottom of the vessel. The heating should be done in a glass flask on a wire gauze over a flame, shaking frequently, and taking care that it does not become heated to boiling point. The solution must be filtered if on cooling it is not perfectly clear—which as a rule only occurs when the ammonium sulphate is not quite pure. As samples of ammonium sulphate vary in value it is advisable to compare, by collateral tests on different specimens of spinal fluid, each freshly prepared solution with a solution already found to be good. The ordinary ammonium sulphate of commerce is useless; that prepared by Messrs. Merck proved excellent. The solutions will keep.

The main condition for practical success in performing the Nonne-Apelt reaction is in the time-limitation for determining the results, for if allowed to stand for any length of time, positive reactions may be shown by normal specimens of spinal fluid too. According to Nonne-Apelt, the results of the reaction exhibit the following degrees: The reaction is negative when the mixture is clear by transmitted light. There is "a trace of opalescence" when the liquid contained in a test-tube

appears clear when held against a dark background and lighted from the front, but is not clear when the light is only allowed to enter the test-tube from above. Opalescence—slightly opalescent, or simply opalescence—is present if the test-tube is not clear when held against a dark background or against the source of light. Still stronger degrees of the reaction are characterized by the appearance of turbidity.

A trace of opalescence occurring within a three minutes' period of observation may be taken as normal, as the expression of the small globulin content normally present in the spinal fluid. After long standing a trace of opalescence generally appears, and in some cases there is a slight opalescence; should slight opalescence appear within three minutes this is an indication of a pathological increase of globulin. The intensity of the reaction is expressed by a number of crosses or by figures, e.g., slight opalescence by $\frac{1}{2}$, marked opalescence by 1; different degrees of turbidity by correspondingly higher figures. Admixture of blood displaces the reaction to the positive side. With a slight admixture of blood the reaction may deliver useful results, but caution must be exercised; a very faint positive reaction is only free from objection when the spinal fluid contains no more than three to four red blood-corpuscles to the small square of the counting-chamber.

The estimation of slight degrees of opalescence requires a certain amount of practice and in some cases even the experienced worker finds it difficult. This is not surprising, for the transition from normal to pathological is gradual. For this reason, a test described by Ross and Jones, which may be readily applied in conjunction with the phase I reaction, provides a welcome support in the estimation of borderland cases. In the Ross-Jones test the spinal fluid and solution of ammonium sulphate are not mixed together, but the specifically denser ammonium sulphate solution is submerged beneath the spinal fluid;

observation is then made whether within three minutes any precipitation occurs at the boundary layer at the plane of contact of the two liquids. The test should be performed as follows: The reagent is drawn into a pipette that is narrow enough to pass into the tubes generally used, which are about 1 cm. wide and 8 to 10 cm. long. The upper end of the pipette is then closed with the finger, and its lower end brought to the bottom of the tube which contains the spinal fluid. The finger is then carefully released and the reagent allowed to enter. The reagent remains below, while the spinal fluid rises (fig. 8). When the pipette has emptied its contents as far as possible, it is removed, after closing again the upper end with the finger so as to prevent the remainder from running out when withdrawing it. In this manner the boundary plane between spinal fluid and ammonium sulphate solution remains quite distinct, and with a positive reaction a delicate, but very plainly visible, white ring, or more correctly a white disc, as thin as paper, appears at the plane of separation of the two liquids. The formation of the ring may be best observed in a dark corner of the room by illuminating the liquid from behind and above with a powerful lamp which is shaded from behind. In the majority of specimens of normal spinal fluid no such ring formation takes place, but in some cases it occurs in non-pathological specimens towards the end of the three minutes' period of observation. A positive result of the reaction with undiluted spinal fluid is thus only to be taken as evidence of an increase of globulin when the ring appears quickly. In case of doubt a decision may be come to by performing the reaction a second time with spinal fluid diluted with an equal volume of physiological saline solution. If this time a ring formation again occurs within three minutes there is a pathological increase of globulin present. (Bertelsen and Bisgaard consider the formation of a ring when the spinal fluid is diluted with twice its

volume as a normal occurrence, which does not tally with my experience.)

For the purpose of combining the Ross-Jones test with that of Nonne-Apelt, it is necessary to underlayer the spinal fluid with an equal volume of ammonium sulphate solution. (Ross-Jones employed 2 c.c. of ammonium sulphate solution and 1 c.c. of spinal fluid.) Owing to the impossibility of completely discharging all the solution contained in the pipette, it is necessary to take into it a greater volume of solution than that of the spinal fluid in the test-tube. For carrying out this reaction I have had 50 graduations, showing $\frac{1}{100}$ c.c. each, marked on the narrow pipettes already mentioned; I employ 0.4 c.c. for the reaction; I therefore draw ammonium sulphate solution into the pipette to somewhere near the graduated marks 45 or 50, and allow the solution to run out until the corresponding mark between 5 and 10 is reached. When the solution has reached that point the pipette is closed again with the forefinger and withdrawn. If, e.g., the pipette has been filled with ammonium sulphate solution to the graduated mark 47, then the outflow of reagent must be interrupted at line 7, and the pipette removed. Observation is then kept for not longer than three minutes for the possible appearance of a ring. After that the liquids are mixed by shaking, and the findings by the Nonne-Apelt test determined. Bisgaard has employed dilutions of pathological fluid with physiological salt solution up to the limit at which ring formation occurs within three minutes after the submersion of the ammonium sulphate solution. This suggestion has already been put forward by Ross and Jones, and is analogous to the total protein estimation according to Brandberg-Pfaundler. I consider this investigation useful, for though it supplies no numerically expressible data of the amounts of globulin present, it gives quite a good picture of the degree of globulin increase; and its relationship to the total

albuminous increase possesses interest.¹ The objection raised by Zaloziecki to the effect that the method is not a pure globulin reaction because all degrees of concentration between 0 and 100 per cent. exist at the plane of contact, and because albumins are also precipitated in higher degrees of concentration than half-saturation with ammonium sulphate, is plausible from a theoretical viewpoint, but without much practical importance, as can be convincingly seen from the presence of a fairly good parallelism between the intensity of the Nonne-Apelt reaction and the estimation by means of this method.

Another reaction in which chiefly globulins are precipitated, and which should be regularly carried out on account of its great sensitiveness and its simplicity, is that of Pándy. The writer performs the test by adding 1 drop of spinal fluid to 1 c.c. of carbolic acid (1 part carbolic acid crystals to 15 parts distilled water), or to a 4 per cent. solution of cresol, or a 10 per cent. solution of pyrogalllic acid. Grahe's procedure is very useful, by which a watch-glass filled with the reagent is placed over a hole cut out of a box lined with black paper and illuminated from below by an electric lamp in a side opening of the box; then with a capillary tube, to allow one drop of the spinal fluid—preferably centrifuged, if there is admixture of blood—to flow into the watch-glass from the margin or into the middle.

Zaloziecki has given practical directions for the preparation of the solution of carbolic acid. 80 to 100 c.c. of pure liquefied carbolic acid is made up to 1 litre with distilled water, well shaken, and placed in the incubator

¹ Bisgaard has given a more accurate determination of the globulin components and the proportions of globulin to albumin in the spinal fluid by determining the total proteins in the unchanged fluid and in the fluid after it has been freed from the globulins by salting out. The quantitative determination of the globulins by centrifugation of the fluid mixed with ammonium sulphate in the tube itself and measurement of the precipitate (Szécsi) has not proved a success in my hands, nor with Bertelsen and Bisgaard.

for a few hours, then allowed to stand at room temperature for several days until perfectly clear. The watery solution which is above the oily carbolic acid is then poured off and kept as a reagent. The solution becomes cloudy at a low temperature, but this cloudiness can be removed by slightly warming it; in uncovered vessels, before an open window, or close to ether, it also becomes cloudy.

In cases on the border line Pándy's reaction is not easy to estimate, as a delicate, gradually formed haziness is still to be regarded as negative. The reagent is, moreover, sometimes too sensitive. With strong reaction a dense, "milky" precipitate is formed; in weaker reactions there is a faint turbidity (turbid, very turbid), which generally increases. The intensity of the reaction is expressed in a similar way to that in the Nonne-Apelt test, by crosses or numbers, e.g., rapidly appearing, slight haziness as $\frac{1}{2}$, moderate turbidity or cloudiness as 1, strong cloudiness as 2, milky turbidity as 3.

On the whole the reaction is more sensitive than the Nonne-Apelt test, i.e., it indicates a pathological increase of globulin in some cases in which the phase I reaction is still negative; but the reverse also occurs in rare cases.

Zaloziecki's statement that with negative Pándy reaction the total albuminous content of the spinal fluid is always lower than one-third per thousand, and that the negative outcome of the test excludes with certainty any pathological increase of albumin,¹ is correct with isolated exceptions; likewise Zaloziecki's other rule, viz., that a positive Nonne-Apelt reaction invariably indicates an albuminous content of more than one-half per thousand.

The practical value of both the reactions here described lies in their being easily carried out. When both reactions are in undoubted agreement, one is in possession of

¹ According to Lange, however, the border line of the normal albuminous content lies much below this.

a satisfactory finding sufficient for the purposes of practical diagnosis. In cases, however, in which the reactions do not permit of sure interpretation, or where questions of research are at stake, or in which continued investigations are desired for the purpose of establishing the effectiveness of a particular therapeutic course adopted, in such cases the quantitative investigation of the total protein content is essential. I carry out such an investigation as a general rule side by side with the Ross-Jones-Bisgaard method of underlaying ammonium sulphate solution, for the performance of several different reactions for albumin affords a good control over a result of such importance, while any occurrence of contradictory findings induces a repetition of the tests.

CHAPTER XIII.

ESTIMATION OF THE TOTAL PROTEIN CONTENT ACCORDING TO NISSEL, AND BRANDBERG-PFAUNDLER. TECHNIQUE OF THE COMBINED APPLICATION OF ROSS-JONES'S AND BRANDBERG-PFAUNDLER'S TESTS. CLINICAL DATA ON THE INCREASE OF PROTEINS.

OWING to the comparatively small amounts of albuminous substance in the spinal fluid, even in the greater number of pathological cases, and owing to the small bulk of fluid as a rule at our disposal for the purposes of investigation, it is not possible to undertake exact quantitative analyses of the total protein content. Such analyses by weight have, however, been carried out in a few cases of meningitis and hydrocephalus, in which larger amounts of fluid can be withdrawn relatively rich in albumin.

Nissel's method has come into extended use owing to its simplicity. It represents a transference of Esbach's method of ascertaining the amount of albumin in urine. Two cubic centimetres of spinal fluid are mixed with 1 c.c. of Esbach's reagent (picric acid one part, citric acid two parts, distilled water 100), and centrifuged for one hour, or, more strictly, until the sediment has been reduced by centrifugation to the smallest possible bulk. The centrifuge tube is graduated to 1, 2 and 3 c.c., and in addition to this at the thin end of the tube is a further graduation of the first cubic centimetre into ten parts, and of the first tenth into hundredths. The amount of albuminous matter is expressed by the number of the line, counting from below, to which the highest level of the sedimented precipitate

reaches. A precipitate, e.g., which reaches to the line that corresponds to 0.04 c.c., is designated as "Nissl 4," one which reaches about midway between 0.1 and 0.2 c.c. as "Nissl 15." (Such high values as the last are rare.) Nissl considered a height of precipitation reaching to the first two or three graduated marks as normal. He worked with a comparatively slow centrifuge run by water power. With a more rapid centrifuge, precipitations higher than 1 to $1\frac{1}{2}$ graduated lines are to be regarded as pathological. In rapid centrifuges one graduation corresponds to a total protein content of about 0.2 per thousand. Even if the centrifugation is always performed in the same machine and the tubes have been graduated expressly from solutions of albumin determined by weight, yet the method contains many sources of error, and Nissl himself stresses its inaccuracy; e.g., obliqueness of the upper level, inequality of the precipitate, varying volume of the precipitate with different varieties of protein, influence of temperature on the volume of the precipitate.¹

I prefer, therefore, with Zaloziecki and Bisgaard, and in opposition to Kafka, not to recommend the method, especially as 2 c.c. of spinal fluid are necessary for it; while, as a rule, a special amount of spinal fluid is not in the least necessary for the much more accurate estimation of the total protein content by underlaying with nitric acid—on the contrary, this reaction can usually be carried out with the fluid employed for the phase I reaction. If, for the purpose of economizing spinal fluid, the amount employed in Nissl's method were reduced, and the bulk raised to 2 c.c. by adding water, it would then be necessary to multiply the height of the precipitate by the dilution factor, which would render the reaction perfectly useless, as this would essentially increase its inaccuracy, thus putting the method out of account for even an approximate quantitative estimation, to say nothing of establishing minute increases of protein.

¹ According to Bisgaard, the error can amount to 100 per cent.

The method of submerging nitric acid, known in the investigation of urine as Brandberg's method, was first employed on a larger scale for the spinal fluid by Pfaundler. Its object is to ascertain the maximum dilution of the spinal fluid at which, after the submersion of nitric acid, a distinct ring formation occurs within three minutes. The reaction is performed with 25 to 30 per cent. nitric acid (the officinal acid, nitric. purum) obtained from a reliable source, and of a specific gravity of 1.15 to 1.2. The spinal fluid is diluted with physiological saline solution. To prepare the dilutions I use the narrow pipette, already mentioned, and mix by repeatedly drawing in and blowing out the liquid.

For urine the rule holds good that the total amount of albumin expressed in parts per thousand is obtained by multiplying the end result of the dilution-series by $\frac{1}{30}$. For example, if a specimen of urine just shows with a twenty-fold dilution a ring formation within three minutes after submerging nitric acid, then it contains two-thirds per thousand of albuminous matter ($20 \times \frac{1}{30}$). As the personal equation varies with different workers, instead of employing the universal factor $\frac{1}{30}$, it is better for each worker to institute comparative investigations in a definite experimental order on a solution of albumin of known strength (determined by weighing), so as to establish the value of the personal equation. (Zaloziecki, e.g., found with his order of experiment that each degree of dilution corresponded to $\frac{1}{60}$ per thousand; with Grahe this factor is $\frac{1}{50}$ per thousand; and in the order which I adopt it amounts to $\frac{1}{40}$ to $\frac{1}{45}$ per thousand for me personally; and I have not experienced any advantage from the employment of a dark box with openings for the test-tubes and illumination from above, as recommended by Zaloziecki and Bisgaard, in preference to the illumination which I use, and described above in dealing with the Ross-Jones test. I have no personal experience of the ingenious apparatus devised by Ameseder for use with the

Brandberg urine test, recommended by Dlabac̆ for the investigation of the spinal fluid. The apparatus has the disadvantage of necessitating large amounts of spinal fluid.) According to the value of this personal equation, and according to the albumin content of the spinal fluid regarded as within the normal by different writers, there emerges a different value for the particular dilution of spinal fluid which represents the border line between the normal and the pathological. While Zaloziecki considers values over $\frac{1}{3}$ per thousand as pathological—corresponding in his researches to a twenty-fold dilution of spinal fluid—Grahe, in agreement with Quincke, is of the opinion that there are border values between $\frac{1}{3}$ and $\frac{1}{2}$ per thousand that should not be taken without exception as pathological; and that only an increase beyond $\frac{1}{2}$ per thousand, i.e., with Grahe corresponding to a twenty-five-fold dilution of spinal fluid, may safely be considered pathological. In my own investigations the formation of a ring with a fifteen-fold dilution, i.e., an albumin content of over $\frac{1}{3}$ to $\frac{2}{3}$ per thousand, proved pathological; while I regard a positive outcome with a ten-fold dilution as within the normal¹; ring formation occurs with a five-fold dilution in all normal cases. The reaction may be performed with a dilution of 1 in 15, if a more accurate determination is unnecessary, as a comparatively rough test for the total protein content side by side with the Nonne-Apelt and the Pándy tests.

If there is an admixture of blood, Lange recommends the following method of control: The total volume of spinal fluid withdrawn is accurately determined, vigorously centrifuged, and the fluid poured off. The sediment of erythrocytes is then dissolved in a small volume of distilled water, the reddish liquid drawn up into a

¹ According to Lange the border line of the normal is to be found with a 6- to 7-fold dilution. He finds in normal cases constant protein values of 0.18 to 0.19 per thousand, and considers a protein content of 0.25 per thousand as indubitably pathological.

capillary tube, and a few drops of distilled water added to the remainder. The total volume of the solvent liquid should be accurately determined, preferably by weighing, and the hæmoglobin content estimated by colorimetric means. In this manner, assuming normal conditions, the percentage admixture of red corpuscles, and from this the corresponding amount of protein in the admixed blood-plasma, may be calculated.

The highest degree of albuminous increase is found in some cases of space-limiting lesions within the vertebral canal, in which coagulation of the whole of the fluid sometimes occurs ; in such cases the albuminous increase amounts sometimes to several parts per cent. Considerable degrees of albuminous increase are also met with in pyogenic meningitic diseases and in hydrocephalus, sometimes up to 1 per cent. and more ; lower degrees occur in the serofibrinous (tubercular meningitis) and the serous forms of meningitis. Medium degrees of protein increase occur in most cases of syphilitic affections of the central nervous system—in paralysis in about 95 per cent. of the cases with an average total albumin content of $1\frac{1}{2}$ per thousand ; in tabes in about 80 per cent. with an approximate average of 1 per thousand. Other diseases of the nervous system and other general diseases are less frequently accompanied by albuminous increase, and exhibit as a rule only low degrees of the same.

The combined investigation of the globulin and the total protein content I carry out in the following manner : A test-tube stand provided with twelve openings holds the small test-tubes to be employed for the reaction (*see* fig. 8). The tubes must be scrupulously cleansed with aqua regia, or concentrated sulphuric acid, &c., and special care must be taken that no soiling be present—as can easily be the case when tubes are in constant use—at that part of the tube at which the formation of a ring is to be observed. The first small tube to the left contains the undiluted spinal fluid, with the name of the patient,

or other sign ; the second tube serves to carry out the Nonne-Apelt reaction. I fill it with 0.4 c.c. of spinal fluid and underlay it with an equal volume of ammonium sulphate solution, and note the results of the Ross-Jones and the Nonne-Apelt tests. I now dilute this mixture with physiological saline solution so as to amount to five times the quantity of spinal fluid taken, viz., 0.4, i.e., by the addition of 1.2 c.c. of physiological salt solution (0.4 spinal fluid + 0.4 ammonium sulphate solution +



FIG. 8.

1.2 saline solution = 2.0). The precipitate from the phase I reaction, even had it been ever so positive, is now completely redissolved, as with such extensive dilution of the ammonium sulphate solution the globulins remain dissolved. From this five-fold dilution I prepare the further dilutions for underlaying with ammonium sulphate and with nitric acid. Measured volumes of the reagents are not necessary in underlaying in the manner described for the Ross-Jones test, for we are now no

longer concerned with the observation of a change brought about by mixing the two liquids, but only with the production of a ring at the layer of contact, which is independent of the volumes of the liquids brought thus together. For underlaying with nitric acid I prepare dilutions in multiples of 5 (i.e., 5-, 10-, 15-fold, &c., dilutions); for underlaying with ammonium sulphate I employ a 5-, 8-, 10-, 12-, 15-fold dilution; I do not, however, always prepare a complete series of the same dilutions in the manner of some writers, but I select the particular degrees of dilution to be next investigated according to the findings of the Nonne-Apelt test. If the Ross-Jones and the Nonne-Apelt tests are negative, then, of course, the further process of underlaying with ammonium sulphate is not applied, and it is usually sufficient to test a 10- to 15-fold dilution with nitric acid. To prepare the dilutions I generally take such amounts of spinal fluid that the total volume of liquid to be submitted to the tests, i.e., the already diluted liquid, amounts to about $\frac{1}{2}$ to 1 c.c. The volume of diluting solution is calculated according to the equation $5 + 5x =$ the required dilution, i.e., in words, how many times 5 must be added to 5 in order to obtain the required number.

If several different specimens of spinal fluid are to be investigated at the same time, it is advisable, after having carried out the Nonne-Apelt test, to note a kind of plan in advance, before preparing each series for further investigation, against each tube on a piece of paper placed under the test-tube stand, of what dilutions are to be taken, and what amounts of spinal fluid and saline solution it is intended to add. If it is desired, e.g., in the first series of tests with a specimen of spinal fluid, to prepare a 5- and 10-fold dilution for underlaying with ammonium sulphate, and a 30- and 40-fold dilution for underlaying with nitric acid; further, with a second specimen of fluid to prepare a 10- to 15-fold dilution for underlaying with ammonium sulphate, and a 40-, 50-,

and 60-fold dilution for the nitric acid test; it should be noted on the paper under the test-tube stand for Series 1 (see fig. 8) below the third tube—5 at the top of the column (= dilution of spinal fluid to be tested), then underneath it—0.5 (= volume of spinal fluid), then 0 (= addition of saline solution); fourth tube—top of column 10, below it 0.3 and 0.3; under the fifth tube—at the top 30, below—0.1 and 0.5; and under the sixth tube—at the top 40, below it 0.1 and 0.7. On the paper under the second stand should be noted in the same manner: At the third tube—top of column 10, below 0.3 and 0.3; opposite the fourth tube—15 at the top and 0.2 and 0.4 below; at the fifth tube—40 at the top and 0.1, 0.7 below; and under the sixth tube—50 at the top and 0.1, 0.9 below; and under the seventh tube—60 at the top and 0.1 and 1.1 below. (In the case of very positive specimens of spinal fluid in which the investigation of such a high dilution as in the last example is rendered necessary, it is advisable to prepare from the 5-fold dilution a larger volume of a 10-fold dilution, and to employ the last for the further degrees of dilution.) After such a plan of investigation has been drawn up, the quantities of spinal fluid noted under the tubes are to be brought into the tubes one after another. Then in the same manner the appropriate volume of saline solution is added. The liquid in the tubes must then be mixed up by passing in the same series from the highest to the lowest degree of dilution. It is not necessary to rinse the pipette each time, blowing it out is sufficient. All the tubes to be treated with ammonium sulphate are then underlaid ("*unterschichtet*"); then all the tubes to be treated with nitric acid, and three minutes after the first tube has been underlaid one can begin reading off the findings, which are to be noted immediately on the paper at the corresponding place.¹

¹ I give all these details intentionally with the object of supplying time-saving hints to the inexperienced worker. With greater experience the details can be modified according to discretion.

There is a certain relationship between the intensity of the globulin reactions and the total protein content in that a stronger globulin reaction generally corresponds to a greater increase of the total protein substances, without, however, a strict parallelism in this respect being present. On the other hand a Nonne-Apelt reaction of the same intensity, i.e., the formation of a ring with ammonium sulphate with the same degree of dilution, can correspond within wide limits to different values for total protein content. Bertelsen and Bisgaard already pointed out that in the spinal fluid of paralysis the globulins had a comparatively larger share in the protein increase than in other diseases—hence the designation “abundant in globulin” (“globulinstark”). These writers found in most of their cases of paralysis a ring production by underlaying with ammonium sulphate in a ten-fold dilution, and with nitric acid in a fifty-fold dilution. If the total protein content after salting out the globulins amounts to over 50 per cent. of the original protein content, the presence of a paralytic lesion is, according to these authors, improbable. Lange places special significance in the ratio of globulin to albumin—the protein quotient. He considers that this ratio is determined by the relation between the endogenous and the exogenous albuminous substance of the spinal fluid. The endogenous protein, characterized by the quotient $1:1$, he regards as the product of cytolysis of the spinal fluid cells; it is found in normal spinal fluid, and in increased amount, but originating in the same manner, in syphilis. Exogenous albuminous substances, i.e., protein that has reached the spinal fluid from the blood plasma, is found in increased permeability, and is characterized by a smaller quotient; this amounts in extreme cases of tubercular meningitis to only $1:10$, in meningitis on an average $>1:5$, and in stagnation of spinal fluid, &c., $<\frac{1}{2}$.

CHAPTER XIV.

A FEW LESS CURRENT METHODS OF ESTIMATING THE PROTEIN CONTENT. COAGULATION OF FIBRIN IN THE SPINAL FLUID.

A NUMBER of other methods of estimating the total protein content of the spinal fluid have been suggested which have not been widely adopted. Among these methods the following are noteworthy:—

Mestrezat developed his “diaphanometric” method out of the boiling test employed by the French in their first examinations of spinal fluid. In a series of tubes are placed 2 c.c. in each tube of a liquid containing a known amount of protein (urine with abundant albumin, determined by weight), diluted with physiological saline solution so as to contain 0.05, 0.1, 0.2, &c., up to 0.8 of albumin per thousand. These are carefully boiled and each tube is treated with six drops of trichloroacetic acid 1:3, again heated and allowed to cool. The tubes are then hermetically sealed and sterilized in the autoclave. Spinal fluid to be tested is treated in the same manner, diluting it if necessary, compared after thirty minutes with the tests in the sealed tubes, and the total protein content thus estimated.

Founded on a similar principle is the method of investigation with Walbum’s albuminometer, in which the spinal fluid is treated with 10 per cent. trichloroacetic acid and then diluted until it coincides in appearance with a sheet of opal glass of a definite degree of turbidity. Two cubic centimetres of spinal fluid are placed in a small graduated tube, the reagent is added as far as a certain line (0.1) and gradually diluted with a 25 per

cent. sodium chloride solution until the turbidity of the opal glass has been reached. The protein content can now be read off directly at the upper level of the liquid in one-tenths per thousand. Should the protein content exceed 1 per thousand it is necessary to take 4 c.c. of the mixture, place it in the small tube again, and dilute as before. The protein content may now be read off, but in ten-fold amounts per thousand as compared with the first examination.

Lange endeavours in the following diaphanometric method, of which he commends the extraordinary precision, to remove the defects attaching to Mestrezat's method, viz., unequal turbidity, and difference of turbidity in the presence of different proportions of globulin and albumin. 0.5 c.c. of spinal fluid is mixed cold with 1.5 c.c. of 10 per cent. sulphosalicylic acid. *Homogeneous* turbidity is produced immediately, which reaches a provisional maximum in one or two hours. If a flocculent precipitate occurs the liquid must be diluted accordingly. The readings must always be taken at the same time within this interval. A solution of agar is employed as a standard, in which barium sulphate, that has been precipitated in finest suspension from a highly diluted solution, is evenly distributed in an amount necessary to produce in the agar a degree of turbidity two or three times as strong as is the case when normal spinal fluid is treated with the above-mentioned reagent. This standard solution is allowed to solidify in the glass wedge of an Autenrieth's colorimeter. For the investigation the spinal fluid is treated with the reagent and placed in the colorimeter vessel; the colorimeter is directed towards an evenly illuminated surface and the wedge moved along until the horizontal lines (drawn with indian ink and a ruling pen on the frosted glass in such a manner that their breadth increases according to the thickness of the wedge) are seen through the wedge and the colorimeter trough with an equal degree of turbidity.

Angistrou makes use of Tanret's reagent for the estimation of the total protein content (mercuric chloride 1.35, potassium iodide 3.32, acetic acid 20, water [ad] 60). The particular degree of dilution in which the reagent just produces a granular precipitate contains 0.5 of albuminous matter per thousand. A method suggested by Weiss and recommended by Kafka is based on a similar principle. The reagent employed is a solution of phosphotungstic acid so prepared that when a liquid containing protein is added to a definite volume of the reagent the first appearance of turbidity corresponds to a protein content of 0.0001 gm. The spinal fluid is added drop by drop until turbidity appears, when the protein content may be readily calculated from the volume of fluid which has been added.

A globulin reaction which is much used in some countries, but which is superfluous beside the Nonne-Apelt and Pándy tests, has been worked out by Noguchi. 0.1 c.c. of spinal fluid is briefly boiled with 0.5 c.c. of 10 per cent. purest butyric acid, then heated with 0.1 c.c. of an n-solution of caustic soda, and boiled for a few seconds. The reaction is positive if a granular or flocculent precipitate is formed that falls to the bottom within three hours. If opalescence or diffuse turbidity appears the reaction is to be regarded as negative. Should the results be doubtful the reaction must be repeated with double the quantities.

Of some interest is Weichbrodt's corrosive sublimate reaction; 0.3 of the reagent (a 1 per 1000 solution of mercuric chloride puriss.) is added to 0.7 spinal fluid in a tube, which is then shaken. A positive reaction may be recognized by different degrees of change in the liquid, e.g., opalescence, turbidity, very turbid, milkiness, &c. It is also possible to perform the reaction by first carefully floating the reagent on the spinal fluid, and observing the formation of a ring. A faint ring then indicates a negative, and only a distinct ring formation a

positive reaction. In general the corrosive sublimate test runs parallel with the globulin reactions, but it is peculiar in that in meningitis it is comparatively weak, while in syphilitic affections of the central nervous system it is in some cases especially pronounced compared with other globulin reactions.¹

The attempts made in a series of chemical researches (Hoppe-Seyler, Reye, Fuld and Spiro, and others) to separate the different globulins by fractional salting out with ammonium sulphate have been applied to the spinal fluid by Kafka and Rautenberg, who give some differential value to the reactions. I do not consider that these theoretically interesting researches are of practical importance. The reactions are arranged in a series of four tubes, in which the first contains 0.5 spinal fluid.

		1st contains 0.5 sp. fluid	...	...	...	+ 0.5	$\left\{ \begin{array}{l} \text{sat. solution} \\ \text{of ammon.} \\ \text{sulphate} \end{array} \right.$	
The 2nd	"	0.6	"	or 0.5 sp. fl.	+ 0.1 aq. dest.	+ 0.4		
" 3rd	"	0.67	"	" 0.5	" + 0.17	" + 0.33	"	"
" 4th	"	0.72	"	" 0.5	" + 0.22	" + 0.28	"	"

There is thus in the first tube a 50 per cent. saturation with ammonium sulphate (the Nonne-Apelt reaction); in the second a 40 per cent. saturation; in the third one of 33 per cent.; and in the fourth a saturation of 28 per cent.²

¹ Eskuchen considers that I under-rate the value of Weichbrodt's reaction. I cannot say that I assign any special importance to it. It is probably correct that it is comparatively feeble with serum-albumin (admixture of blood, meningitis, stagnation of fluid); and comparatively pronounced in lesions in which the parenchyma is greatly injured; and that accordingly, as against the Nonne-Apelt test, the endogenous protein is more precipitated by it. But in this respect it cannot at all be compared with the gold test, which is not surprising in view of its coarseness from a chemical standpoint.

² The reaction should not, of course, be performed by diluting the spinal fluid, e.g., by arranging a series of 0.4 spinal fluid + 0.1 aq. dest., 0.33 sp. fl. + 0.17 aq. dest., and 0.28 sp. fl. + 0.22 aq. dest., and then adding to each tube 0.5 ammonium sulphate solution. This would not produce a fractional precipitation, but would cause precipitation in each tube of the total globulins contained in the spinal fluid therein (i.e., 0.4, 0.33, 0.28), in other words, the Nonne-Apelt test would thus be applied to different dilutions of spinal fluid.

The tubes are well shaken and allowed to stand for sixteen to twenty-four hours, and any precipitations which occur are noted in four or five hours and at the end of the period of observation. Kafka afterwards complicated the method still further by centrifugalizing in graduated tubes and observing the height of the precipitate. The question is not yet decided whether in the given concentrations contained in the second to the fourth tubes different globulins are actually precipitated, such as pseudoglobulin, euglobulin, fibrinoglobulin or fibrinogen. While in most of the organic diseases of the central nervous system that are accompanied by an increase of globulin, only the phase I reaction is positive, but with a lower ammonium sulphate concentration nothing is precipitated, it is found that in fresh cases of cerebro-spinal syphilis, and in most cases of paralysis, precipitations occur with 40 per cent. saturation, in paralysis frequently with 33 per cent.; in acute meningitic lesions (syphilitic and non-syphilitic) a precipitation generally occurs with a 33 per cent. saturation, and often with a saturation of 28 per cent.

A method devised by Hudovernig is also founded on fractional precipitation. To 1 to 2 c.c. of spinal fluid are added 1 to 2 drops of a 3 per cent., 2 per cent., and 1 per cent. solution of sulphosalicylic acid; the method is not of any practical value.

Of theoretical interest is the hydrochloric acid reaction of Braun and Husler, which has the distinction of being specially pronounced chiefly in acute lesions (meningitis), but is also positive in a large number of cases of paralysis, while in the majority of syphilitic affections it is negative.¹

¹ Riese even found (*Zeitschr. f. d. ges. Neurol. u. Psych.*, 1918, vol. xxxix) a positive reaction in all cases of undoubted paralysis, which does not conform with my experience. The reaction has been brought into a certain connection with the hæmolysin reaction, and with the passage of complement into the spinal fluid. Sachs and Altmann found that the so-called "intermediary substance" of the complement

The reaction is carried out by adding 1 c.c. at a time of an $\frac{n}{300}$ -solution¹ of hydrochloric acid to 1 c.c. of spinal fluid. (The reaction can also be performed with half these amounts.) If neither opalescence nor turbidity are produced by the addition of 5 c.c. of the reagent, the reaction is negative. The test is usually positive in a short time, but it should be left standing for two hours. In doubtful cases the reaction, if positive, can be employed with others for the diagnosis of paralysis.

Zaloziecki holds that the reaction is nothing more than the acetic acid reaction of Moritz and Rivalta for distinguishing between exudates and transudates. (Eskuchen thinks that the proteins that are precipitable by means of acetic acid probably belong to the albumoses, which are absent from normal spinal fluid, but occur in certain pathological lesions.) Zaloziecki applies Moritz's test to the spinal fluid by adding 5 per cent. acetic acid dropwise to 2 c.c. of spinal fluid—the amount of fluid can be reduced if capillary tubes with smaller-sized drops are employed. It is then noted whether opalescence or turbidity occurs within one to two minutes. A "trace of opalescence" is regarded as a negative reaction.

Among the proteins of the spinal fluid special reference must be made to fibrinogen, as the appearance of a delicate fibrin clotting is of considerable significance in the diagnosis of tubercular meningitis. In interpreting the clot formation special attention must be paid to the following (cf. Zaloziecki): The more fibrinogen the spinal fluid contains the larger the clots, and they are the more visible the quieter the fluid is allowed to

is precipitated by a definite hydrochloric acid concentration. As the precipitate is redissolved in a slight excess of hydrochloric acid, a large amount of the reagent should not be immediately added when performing the Braun-Husler reaction, but it should be added a cubic centimetre at a time, so as not to go beyond the optimum of the precipitation.

¹The solution should be preserved in bottles made of Jena glass, as ordinary glass gives off alkali.

stand ; if it is shaken, small flocculent masses are formed which become deposited on the side of the vessel, and can be easily overlooked. It is of importance to make repeated examinations during the twenty-four hours subsequent to removing the spinal fluid—not leaving the examination until this period has elapsed—for it is possible for the flocks to disappear again. Lange recommends allowing the spinal fluid after withdrawal to stand at least an hour in a water-bath or in the incubator. Small quantities of fibrin which may appear after standing a longer time have, he thinks, no differential value. Centrifugation of the fluid is not permissible, because the cells present in the fluid are of importance in the production of the clots. The clotting can be hastened by the addition of fresh serum, which contains fibrin-ferment (Mestrezat)—the fibrin originates, of course, from the co-operation of fibrin-ferment and fibrinogen—but this is not as a rule necessary in the case of spinal fluid, as sufficient ferment is usually present ; the addition of ferment, moreover, excludes the employment of that portion of spinal fluid from further investigations.

In tuberculous meningitis the presence of a usually moderate amount of fibrinogen, but in which a part of the fibrin probably disappears by fibrinolysis, leads to the characteristic spider's web appearance of the clot ; while in pyogenic meningitis, in which fibrin clotting also regularly occurs, a dense, mucose flock arises which rapidly falls to the bottom and is not again recognized. A slight separation of fibrin also occurs occasionally in paralysis (Kafka considers, wrongly in my opinion, as opposed to Zaloziecki, that this is only the case when a slight admixture of blood is present) ; it can only be seen by rubbing the side of the test-tube with a platinum needle (Zaloziecki), when tiny flocks stick to the point.¹ I was

¹ I called attention many years ago to the occurrence of clot-formation, which I first came across in examinations in the counting-chamber. The slight clotting which occurs in paralysis is not of any practical consequence.

also able to observe a slight clotting in a few cases of uræmia. That clotting can easily occur with an artificial admixture of blood, and that this is of differential value in diagnosing between this and pathological hæmorrhages, has been already mentioned when discussing admixture of blood.

The highest degree of fibrin clotting is met with in some cases of occlusion of the subarachnoid space by a tumour of the cord, by caries of the vertebræ, occasionally also by meningitic adhesions of varying degrees, in different forms of meningitis, in gummatous syphilis, in alcoholic polyneuritis with meningo-medullary lesions, &c.; these clottings, however, are vesiculiform ("blasenförmig"), not in the form of branching threads, as is the case in tubercular meningitis. In a few of these cases it can come to a jelly-like clotting of the whole fluid, that appears either immediately or which may require a longer period, up to several hours, to develop to the fullest extent. The combination of this symptom—the so-called *coagulation en masse*—with xanthochromia, is called, after its discoverer, Froin's syndrome. (It may be said in passing that the same changes take place in the fluid which is present in closed cysts of the brain.) Lange rightly stresses the fact that the xanthochromia in these cases is only of secondary importance, as it invariably occurs with massive clotting, and can also be always proved to be present in clotting of lower degree, though at times only with the aid of accessory methods.

CHAPTER XV.

THE GOLD REACTION. THEORETICAL
CONSIDERATION OF THE COLLOIDS.
PREPARATION OF THE COLLOIDAL GOLD.
PERFORMANCE OF THE GOLD REACTION.

LANGE'S introduction of the gold reaction represents a substantial advance in the methods of investigation of the spinal fluid. It is founded on the different kind of precipitation of colloidal gold by different specimens of pathological spinal fluid. Colloidal solutions occupy an intermediate position between the apparently homogeneous solutions of the so-called crystalloids¹ and the mechanical suspensions of coarser particles, such as emulsions. The "periods" of the coarse dispersions are larger than 0.1μ ; the particles can, therefore, be rendered visible in the microscope; owing to their size, moreover, they will not pass through filter paper, nor, of course, through membranes. The periods of the molecular dispersoids are smaller than 1μ ($= \frac{1}{1000000}$ mm.); they are not capable of resolution by the microscope, and will pass through membranes as well as through filters. Colloids lie with reference to the dimension of their periods, and to other characters, in the middle of these "disperse systems." The dimensions of their periods are between 0.1μ and 1μ . Their resolution is not possible with the ordinary microscope, the resolving power of the ultra-microscope being necessary to render them visible; and they will pass through filter paper, though not through membranes.

¹ Graham (1861) was the first to separate crystalloids from colloids by dialysis (i.e., by the employment of a suitable membrane, a so-called dialyser) by which the crystalloids passed through the membrane, leaving the colloids behind.

Colloids thus possess the character in common with coarse mechanical suspensions that they are not dialysable; and with molecular solutions that they will pass through filter paper. Between these three systems there are, of course, no distinct boundaries; they are, on the contrary, interconnected in various respects by gradations.

Not only can solid particles be dispersed colloiddally in a liquid—e.g., gold sol—but one liquid can be colloiddally dispersed in another, or a gas in a liquid, e.g., froth; or a liquid in a gas, as in mist; or solid particles in gas, e.g., smoke; gas within a solid substance, e.g., meerschaum; a solid in a solid substance; thus ruby glass is a colloiddal dispersion of gold in glass; blue rock salt one of metallic sodium in common salt.

Colloids can be prepared in various ways, by chemical, electrical, and mechanical means, but according to two different methods; either by raising the degree of dispersion of a mechanical suspension—the dispersion method, or by reducing the degree of dispersion of a molecular solution—the condensation method. Colloiddal gold, otherwise known as gold sol, as employed in reactions with spinal fluid, is prepared by a chemical condensation method.

Most colloids are of a very unstable character, i.e., they may be readily converted in various ways—chemical, mechanical, electrical—into coarse dispersions, generally so that the precipitation which results, the so-called gel, can no longer be brought back into the colloiddal state; this process is known as coagulation or pectization.¹ On the other hand, the precipitating effect, e.g., of salts on colloids, can be obviated by the agency of “protective colloids,” such as certain albuminous substances.

¹ So-called reversible colloids also exist, i.e., colloids whose residue after drying by evaporation can be converted into a colloid again by the addition of water: such dry residues are known as solid hydrosols, e.g., gelatine, glue, gums. The metallic colloids, though generally irreversible, can be changed into reversible colloids by combining them with certain reversible colloids, the so-called protective colloids.

Lange, in applying his reaction to the spinal fluid, set out from the fact that normal spinal fluid, when diluted with a sodium chloride solution of definite concentration, produces no precipitate in the gold sol, while samples of pathological fluid, diluted with the same sodium chloride solution, give rise to a varying precipitate. In the complicated conditions which ensue from the interaction of the albuminous colloids of the spinal fluid, the gold sol, and the electrolyte (sodium chloride), changes are brought about in the gold sol which differ in two directions : (1) The degree of precipitation, i.e., of lowering of dispersion, varies, which is expressed in the gold sol by a change of colour. While the colloidal gold solution has a deep red colour—differing here, however, according to the state of division of the particles of gold—with increased dimensions of the gold particles its colour undergoes changes to reddish-violet, violet, blue, bluish-white (it is also possible to set up somewhat different scales of colour), until with complete precipitation the colour of the supernatant liquid is discharged, it appears “white,” and the particles of gold are deposited as a black precipitate at the bottom of the test-tube. (2) The precipitate exhibits differences in that it does not invariably set in with equal dilutions of the spinal fluid to which is added the gold sol, for which reason a geometrical series of dilutions of the spinal fluid is employed in performing the reaction.

Lange employed a sodium chloride solution of 0.4 per cent. concentration as the highest that will effect no change in the gold sol. Kafka points out that in some cases a 0.3 per cent. solution of sodium chloride, in others one of 0.5 or 0.6 per cent., represents the last concentration which produces no decolorizing effect. He therefore recommends a preliminary trial to be made with 1 c.c. of an ascending series of sodium chloride solutions from 0.2 to 0.6 per cent. + 5 c.c. of gold sol. Lange does not approve of such a preliminary trial, as he considers that only those gold solutions are of any

value which are absolutely unchanged by spinal fluid with normal protein content (0.18 to 0.19 per thousand)—for the reaction is expressly and empirically arranged with this object in view—while every increase of protein, even the minutest—0.21-0.22 per thousand—must give a plainly discernible positive reaction.¹ The saline solution should be freshly prepared; weighed quantities of salt, or a 10 per cent. solution (10 grm. NaCl in 90 c.c. aq. dest.) can be kept in readiness.

The gold test is very simple to carry out; not so the preparation of the reagent, and hence a series of modifications of Zsigmondy's original method have been put forward from time to time, which all yield serviceable solutions provided the details of technique are all carefully attended to. Such solutions are deeply purple-red in colour, translucent with transmitted light, and showing with reflected light a smoke-grey lustre. Very bright solutions are not good, nor solutions which strongly tend to a blue tinge. Solutions with a very faintly bluish hue sometimes give quite good results, and one or two writers even recommend working with a red solution and a slightly bluish solution side by side. As, however, solutions which have a good appearance are not always serviceable, it is necessary always to compare the effectiveness of new solutions with solutions that have

¹ Brandt and Mras (*Zeitschr. f. d. ges. Neur. u. Psych.*, vol. lxxvi, 1922, p. 529) reject the preliminary trial with sodium chloride on the ground that the alteration of the sodium chloride concentration changes the curve in conformity with law ("gesetzmässig") in that by increasing the sodium chloride concentration the curve-maximum wanders to the right among the higher spinal fluid dilutions, while with a decrease of the saline concentration the maximum is displaced to the left, towards the higher concentrations of the fluid. Kafka contests this change "according to law." In their researches these writers find, furthermore, that in all changes of the spinal fluid due to syphilis the reaction maximum is with a dilution of the fluid of 1 in 80, and that such a precipitation-maximum is always shown in normal spinal fluid. They found that all solutions proved useless which failed to exhibit this sensitiveness with normal spinal fluid (cf. Lange's remarks cited above).

been tried, and to test them with specimens of spinal fluid of known character—not merely with a paralysis and a normal fluid, but also with a very feebly positive one. The gold sol should be kept well stoppered and in a dark place, but if possible not longer than a few weeks. The liquid should be shaken before using, owing to the fine sediment which is deposited after it has been standing some time.

As to the preparation of the solution, I have obtained on an average just as good solutions with Zsigmondy's original method of reduction with formalin as with the methods which employ dextrose as the reducing agent.

Zsigmondy's directions are as follows:—

“25 c.c. of a solution containing 0.6 [gram.] acid chloride of gold to the litre are diluted with 100 to 150 c.c. of water, then treated with 2 to 4 c.c. of a 0.2-normal solution of potassium carbonate or bicarbonate and heated to boiling point. (The amount of alkali and the degree of dilution are dependent on the character of the distilled water; if blackish-violet tints are obtained instead of red, the solution must be diluted more, and more alkali must be taken.) Directly after the solution has boiled it is removed from the flame and 4 c.c. of a solution of 1 part freshly distilled formic aldehyde in 100 parts of water are quickly added to the boiling gold solution by degrees, vigorously stirring the solution all the time. (Only the formaldehyde which passes over between 97° and 100° C. is collected and afterwards employed. It is sometimes necessary to dilute the solution still 3- or 4-fold.)”

Lange adds 10 c.c. of a 1 per cent. gold chloride solution and 10 c.c. of a 2 per cent. potassium carbonate solution to a litre of water, heats it to the boiling point, then quickly adds, in portions at a time, 10 c.c. of 1 per cent. formalin (1 per cent. of the solution usually sold), stirring vigorously.

A number of processes for the preparation of the gold

sol by means of glucose were subsequently recommended. According to Lange this method was first described by Faraday. Lange is not in favour of preparing the sol with glucose as it will not keep unaltered.

Eicke's directions are as follows: 1 litre of freshly distilled water is heated to the boiling point with 10 c.c. of a 1 per cent. gold chloride solution and 5 c.c. of a 5 per cent. solution of glucose. (It is better to take half amounts.) Immediately the solution boils add drop by drop a 5 per cent. solution of potash until the boiling liquid has assumed a deep red colour, which will require about 3.6 to 4 c.c. of the solution of potash.

Oetiker heats 1 litre of water with 5 c.c. of a 5 per cent. glucose solution and 10 c.c. gold chloride solution to 90° C., and immediately adds, without putting out the flame, 12 c.c. of a 2 per cent. solution of potash, all at once.

Schaffer also considers a temperature of 85° to 90° C. to be the most suitable. He gives the following directions: Into a Jena glass flask with a wide, short neck and a capacity of 750 c.c., place 500 c.c. of freshly distilled water. Heat on a wire gauze without asbestos to a temperature of 60° C. (introduce thermometer), then add from a pipette 5 c.c. of 1 per cent. gold chloride solution and 2 c.c. of 5 per cent. solution of potassium carbonate. The liquid is then heated to 85° C., during which it is shaken two or three times, the thermometer removed, the flask taken from the flame and again well shaken. As the neck of the flask is rather hot, it should be held with a small cloth. Just before removing the flask from the wire gauze, 2.5 c.c. of a 5 per cent. solution of glucose is dropped from a pipette, held in the left hand, slowly into the middle of the solution, taking care that none of the solution of glucose touches the hot sides of the flask, and well shaking the liquid during the operation. The flask is now continually shaken over the wire gauze until the desired colour appears. As a rule the solution first

becomes pale pink and almost immediately afterwards dark red. If properly done the colour appears soon after adding the solution of glucose. But if it should not appear, a drop or two of the solution of potash may be added, without, however, interrupting the vigorous shaking.

Weigeldt points out that the amount of grape sugar added is immaterial, but that the decisive factor is the proportion of potash to gold chloride, or the degree of neutralization—(the gold chloride is acid)—and that for this reason it is not possible to determine the necessary amount of potash once for all, owing to the varying quality of the gold chloride. He suggests a provisional preparation of only 100 c.c. of gold sol (I have followed this plan for years), and then to prepare at most 300 to 400 c.c. Weigeldt adds to 100 c.c. of water, immediately after lighting the Bunsen, 0.25 c.c. of 10 per cent. gold chloride solution and 0.60 c.c. of 5 per cent. solution of potash. This is well shaken and heated until the first large bubbles begin to rise, which corresponds to a temperature of about 95° C. (I agree with Weigeldt that the strict observance of the temperatures directed by Schaffer has no importance.) The flame is now removed, the solution well shaken, and 0.3 c.c. of 5 per cent. glucose solution added with a pipette whilst shaking. The flask must now be continually shaken until the reduction has taken place from pink to reddish-violet, yellowish red and reddish purple. If the change of colour to pink has not begun within three to five minutes, a drop or two of potash solution must be added, shaking all the time and continuing to shake; this addition of potash may be repeated if necessary. Custer recommends making a preliminary trial in which the amount of potash necessary to neutralize the gold chloride is determined by means of phenolphthalein as an indicator test.

Neufeld describes a method which he says is practically always successful. Dissolve 1 grm. of yellow gold

chloride crystals and 2.5 gm. of potassium carbonate in a flask with 50 c.c. of freshly distilled water. This solution will keep and may be held in reserve. To one litre of distilled water add 5 c.c. of a 5 per cent. solution of glucose and heat to the boiling point. After it has boiled vigorously, add 1.5 to 2 c.c. of the gold-potash solution and continue to add this solution drop by drop until the smallest change of colour sets in. Then continue to add the drops still more slowly until the solution has become a deep violet colour; at the same moment the gas tap is held with the hand in readiness to be turned off as soon as the purple-red colour appears. The preparation should be undertaken before a window by daylight, so as to be able to observe the colour of the liquid accurately by transmitted light. Beakers are better adapted to the purpose.

In former years I usually obtained good results with some of the methods here described, but noticed now and then, as have most writers, that for some unaccountable reason they all failed. For the successful preparation of the solution it is important to observe the following:—

(1) The purity of all chemicals employed. A good gold preparation is Merck's aurum chloratum crystallisatum flavum. The gold chloride is put up in glass capsules containing 1 gm. It should be dissolved by filing and breaking off the end of the capsule, and washing the chloride out with a definite volume of doubly distilled water—according to whether a 10 per cent. or a 1 per cent. solution is to be prepared. If the solution is not quite clear owing to particles of glass or other impurities, it must be passed through a filter which is moistened with redistilled water. It is not advisable to preserve the gold solution too long. The solution of potash (potass. carb. puriss.) may be preserved in a sterile condition for several weeks, but the solution of glucose (dextrose puriss. free from water) must be frequently renewed. If these

chemicals are to be stored for any length of time, calcium chloride must be placed at the bottom of the vessels in which they are kept, as they are hygroscopic.

(2) The water must be of especial purity. Writers on the subject generally insist on the employment of freshly redistilled water, but Weigeldt rightly remarks that redistilled water may be kept for months in Jena glass flasks, and that if certain precautions are observed once-distilled water suffices for the preparation of a gold solution of perfectly good quality. The distillation retort must be of Jena glass; uncooled steam must be allowed to pass through the tubes for ten minutes before and after use each time for the purpose of sterilizing and cleansing. The first 200 c.c. of water must not be used, and the distillation must stop when about four-fifths of the contents of the retort has passed over.

(3) All utensils which have come into contact with the gold sol must be well cleaned after use, especially the flask in which the reduction has been effected; but also test-tubes, pipettes and the tubes for collecting spinal fluid, also the thermometer, if one has been used, must be cleaned with aqua regia and well washed with water, and finally with the purest distilled water.

(4) The flask in which the gold sol is prepared, as well as the vessels in which the gold sol and the distilled water are kept, must be of Jena glass. (Other kinds of glass give off alkali, which can impair the process of preparation.)

(5) The cannulæ for puncturing must not be boiled with soda, but only in distilled water; it is still better to jet ether and alcohol through them and dry sterilize.

The gold test is performed as follows: A test-tube stand is provided with twelve tubes; for meningitic cases a larger number, say sixteen tubes, is preferable. In the first tube is placed 0.2 c.c. of spinal fluid, then 1.8 c.c. of a 0.4 per cent. sodium chloride solution, or of the strength which has been determined upon in the preliminary trial. In all the following tubes is placed

1 c.c. of the same sodium chloride solution. Now from the tenth dilution of spinal fluid contained in tube No. 1 (0.2 spinal fluid + 1.8 salt solution) a geometrical series of dilutions for the other tubes is made by taking 1 c.c. of liquid from the first tube and placing it in the second tube, 1 c.c. from the second tube into the third tube, and so on until the last tube but one is reached (the eleventh or fifteenth tube) from which 1 c.c. is also removed, but instead of putting it into the last tube it is thrown away. Before each transfer of liquid from one tube to the next the liquids must be thoroughly mixed. After this procedure there will thus be present in each tube a volume of 1 c.c. of liquid, and in tube No. 1 a dilution of spinal fluid as 1 : 10, in tube No. 2 as 1 : 20, in tube No. 3 as 1 : 40, &c., up to a 10,240th dilution in the eleventh tube, and a 163,840th dilution in the fifteenth tube. The last tube, which serves only for purposes of control, only contains 1 c.c. of the saline solution. Into each tube, including, of course, the control tube, is now added 5 c.c. of the gold sol—according to Weigeldt 2 to 3 c.c. is sufficient—and the contents of each tube vigorously mixed together in a uniform manner for all investigations, viz., by holding the tube with the thumb and forefinger (not by closing with the thumb and inverting) and shaking three times.¹

The reactions should be read off three times, viz., at the end of five minutes, thirty minutes, and twenty-four hours. The tubes are left to stand at room temperature. The findings must be noted each time, either by writing down the changes observed in each tube expressed by the colour scale employed, or by rows of numbers from 1 to 6 in the order of the tubes, e.g., 6 6 6 6 5 4 2 1 11 (i.e., first to fifth tubes clear, sixth tube bluish, seventh tube

¹ There is no objection on principle to working with half amounts. But wide enough tubes must be employed for the reaction, so that no difficulty arises in shaking them; the amount of fluid therefore must not be too small.

blue, eighth tube reddish violet, ninth to eleventh red) ; or, finally, by plotting the results of the reaction on a chart (*see* fig. 9), in which the degrees of dilution are marked on the abscissa, and on the ordinate the degree of precipitation of the gold sol.

The differences of colour are most distinctly discernible by diffused daylight ; with artificial light a sheet of white paper should be held behind the tubes. The type of the reaction is usually plain at the first reading, and it becomes accentuated at the second. As a rule there are no essential differences between the second and third readings of the reaction. In the control tube the colour of the gold sol should not have undergone any change.

CHAPTER XVI.

CLINICAL DATA ON THE GOLD REACTION.
SUBSTITUTES FOR THE GOLD REACTION.

VERY soon after its discovery the gold reaction became extensively adopted, especially for the investigation of syphilitic spinal fluid. The numerous verification tests to which the reaction has been put have demonstrated its undoubted value, although the full significance of some of the details has yet to be cleared up.

The spinal fluid of persons with healthy nerves and of those with functional nervous disorders produces no change in the gold sol; slight changes in the third and fourth tubes, however, are not to be regarded as pathological. (Lange, to be sure, objects to such findings; but in view of the difficulties attached to the preparation of the reagent, the decision to exclude gold sols which exhibit slight changes with normal spinal fluid will not be so easy.) The changes in the gold sol produced by pathological spinal fluid are divided into two groups, according to their occurrence in the higher or lower dilutions of the fluid; viz., precipitation in the first half of the tubes, i.e., in the stronger concentrations of spinal fluid, are typical in syphilis; precipitations in the middle tubes, or in the second half, i.e., in the higher dilutions of spinal fluid—the so-called “displacement to the right”—are characteristic of an acute inflammatory affection of the meninges, or of an admixture of serum (Lange. “communication symptom”). According to Lange, with small protein content a displacement by one tube proves the entrance of blood-albumin into the spinal fluid. Also in the syphilis group the strongest concentrations

($\frac{1}{10}$ and $\frac{1}{20}$) of spinal fluid do not exhibit the greatest tendency to precipitation; with those specimens which show a moderately strong gold reaction the most marked changes are found in the dilutions $\frac{1}{40}$ and $\frac{1}{80}$, i.e., in the third and fourth tubes.¹

There are certain differences in the reaction within the various syphilitic groups, though they are by no means absolutely characteristic—merely that certain types of curves preponderate numerically in certain groups of diseases. A type of this nature is represented by the so-called “paralytic curve” (see fig. 9, the lower, thick line), i.e., a great alteration, amounting to complete precipitation, of the gold sol in the first six or seven tubes (sometimes only in five tubes, at times even in nine), and in the next tubes the curve passes with a sudden, steep ascent into the normal.

In the “paralytic” curve the precipitation is also complete in the first tube, i.e., in the dilution of spinal fluid 1:10, but it sometimes occurs somewhat later in this tube than in the following tubes. In paresis the gold reaction is nearly always positive, and the typical curve is shown in the great majority of (N.B. untreated) cases. This last, however, is not always the case, and slighter

¹ Lange regards this precipitation as an increase of the tendencies to precipitation present in normal spinal fluid, which, however, do not make their appearance in the conditions of investigation he lays down. The qualitative conformity of the findings is due to the protein quotient being the same as in normal fluid, viz., 1:1, because the protein has the same origin in both cases (endogenous protein). Lange interprets the paresis curve as the expression of a combined albuminous increase, i.e., endogenous plus exogenous proteins.

Lange, on principle, accordingly places the *syphilitic indentation* (of the curve) in opposition to the right displacements; while it has been usual to contrast the precipitations in the *syphilitic zone* with those obtaining in meningitis. Löwy, Brandt and Mras thus speak (*Med. Klinik.*, 1921, Nos. 7 and 8) of a vascular (“luetetic”) type (syphilis, typhus, encephalitis, &c.), and a “meningitic” type; Goebel of a paralytic type (to which multiple sclerosis also belongs) and a blood-serum type, which is referable chiefly to injury to the vessels, mainly those of the vascular membranes of the brain and cord from congestion or inflammation.

precipitations sometimes occur which resemble those which are the rule in other syphilitic affections. In the spinal fluid of paralysis the precipitation usually occurs very rapidly, so that the type of curve is distinctly shown after a few minutes and very little increase in the changes occurs afterwards.

Somewhat smaller average, but likewise constant, changes are found in syphilis with marked nervous lesions (cerebral, spinal, and cerebro-spinal syphilis), viz., changes in the first half of the tubes which generally do

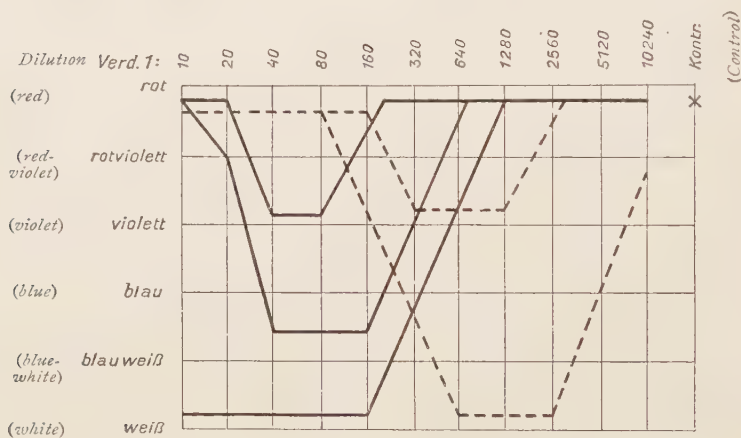


FIG. 9.

not extend to complete precipitation, and which reach a maximum in the 3rd to the 5th tubes, i.e., in the dilutions from $\frac{1}{40}$ to $\frac{1}{160}$ (see fig. 9, middle thick line). Occasionally, however, particularly in fresh cases, the spinal fluid specimens in this group of diseases exhibit typical paralytic reactions, that are sometimes only peculiar in being slower to make their appearance than is usually the case in paralysis. On the other hand, in this group of diseases, slight changes of the gold sol occur in a series of cases which are chiefly limited to the dilutions 1:40

and 1:80, but which are, however, often exhibited to a less degree in the neighbouring dilutions.

Less characteristic are the changes which occur in *tabes dorsalis*. The majority of cases show precipitations of greater or less degree (violet to bluish) in the series of dilutions in which they mostly prevail, i.e., in tubes 3 and 4; in a few cases the reaction is negative. Also syphilis without objective neurological symptoms shows a positive gold reaction in a large number of cases—according to Kyrle and others, in two-thirds of all cases—and in a certain number already in the primary stage. Lange found a positive reaction in syphilis I and II in 64.5 per cent. of cases; Kyrle, Brandt and Mras in 10 per cent.; Weigeldt in 64 per cent. (and in a more extensive case-statistic in 47 per cent.), the last-named in 5 out of 10 cases already in the first stage. Fröhwald observed it in seronegative syphilis I, as the first pathological sign in the spinal fluid. In congenital and tertiary syphilis, positive gold reactions are, in contrast to the other spinal fluid reactions, of frequent occurrence. In cases of syphilis in which the nervous system is not involved, in the majority of cases only slight alterations are shown (1:40 and 1:80, violet to blue)—the so-called syphilitic indentation (“Lueszacke”) (see fig. 9, upper, thick line); but in this group, too, stronger precipitations towards the type of the parietic curve are not infrequently met with.

Slight displacements to the right, i.e., changes in the direction of the meningitic type, are occasionally found in early cases of syphilis with nervous lesions (early syphilitic meningitis, neuro-relapse, &c.). In such cases, however, the types of curve associated with paresis and cerebro-spinal syphilis may sometimes be observed.

The gold reaction not infrequently shows a change after specific treatment, and after antifebrile treatment, in the sense of a displacement “to the left and upwards,” i.e., a decrease of the change in a few tubes with lower concentration of spinal fluid, and a smaller degree of

precipitation. Even general paresis is subject to a certain influence under treatment, the extent of which, however, does not reach that observed in cerebro-spinal syphilis. A completely negative reaction will hardly ever be attained in general paresis, and but rarely in cerebro-spinal syphilis, and then the reaction usually shows a tendency to return. And after effecting a cure in the other kinds of spinal fluid alterations due to early syphilis, the gold reaction sometimes proves specially resistant (Kyrle).

The characteristic of the "meningitic curve" is the absence of changes in at least the first four tubes. In tubercular meningitis there is often only moderate precipitation in tubes 7 and 8 ($\frac{1}{820}$ and $\frac{1}{640}$ dilutions)—see fig. 9, upper interrupted line. But occasionally there are stronger and more extensive precipitations, and in pyogenic meningitic affections changes occur in the 5th to 8th, 5th to 10th, 6th to 11th tubes, and even beyond this up to the 15th tube (see fig. 9, lower interrupted line). In cases of non-syphilitic meningitis with positive Wassermann reaction in the spinal fluid, and with positive as well as negative serum reactions, on performing the gold test a displacement to the right was observed (Eicke); on the other hand, in exceptional cases of non-syphilitic meningitis a reaction in the syphilitic zone occurs.

A noteworthy positive gold reaction of the syphilitic type is also found to occur very frequently in multiple sclerosis and in epidemic encephalitis. Changes in the gold sol are also occasionally met with in epilepsy, schizophrenia, syringomyelia, and in other organic affections. I am able to confirm the findings of Stern and Poensgen as against Eskuchen, that typical displacements to the right are not very infrequent in tumours of the brain and cord, and in cases of caries with compression; this also applies to uræmia and cerebral abscess.

It is of interest that an admixture of blood in the

spinal fluid can also occasion changes of the right displacement type; these usually consist of slight, but sometimes pronounced, precipitations in the 7th and 8th tubes ($\frac{1}{840}$ to $\frac{1}{1280}$), or even in the 6th to the 10th tubes. The addition of inactivated syphilis-serum to a negatively reacting spinal fluid only produces the normal serum effect, but no syphilitic curve (Eicke). The syphilitic curve is as a rule not disturbed by an admixture of blood; but in some cases in which the serum is also productive of changes in the regions more to the left, i.e., in which a double maximum arises from the plasma admixture alone, the admixture of blood can occasion certain difficulties in interpreting the reaction.

The differential value of the gold reaction thus lies in the region of syphilis diagnosis. A positive result can sometimes give support to the differentiation between cerebral syphilis and cerebral arterio-sclerosis. In differentiating between cerebro-spinal syphilis and multiple sclerosis, only a negative finding can be of value in favour of the latter. And in situations in which progressive paralysis may be in question, the occurrence of a slightly positive gold reaction should rather be taken to weigh against this diagnosis, than as affording support to its interpretation as a "paralytic" curve, which is also found to occur in cerebral syphilis. A rapid improvement in the reaction subsequent to treatment also speaks against paralysis.

Some writers credit the gold reaction in syphilis with a certain prognostic value. Thus the outlook for syphilitics with no nervous symptoms, but with a strong positive reaction, is regarded as grave (Kyrle); it is also said to be an unfavourable sign if the reaction increases in intensity in cases of syphilitic disease of the central nervous system already present (Schacherl).

Different views have been expressed on the essential nature of the gold reaction. Lange assumed that the variations in precipitation depend on the different pro-

portions of the various proteins present in the spinal fluid; and he was able to show experimentally that different curves were obtainable from different mixtures of protein substances. Others on the contrary think that the reaction is referable to substances resulting from pathological conditions; thus Matzkewitsch traces the reaction to the precipitating effect of peptones; Neufeld regards it—evidently on erroneous assumptions (Weigeldt)—as a consequence of the reaction between thrombin and antithrombin, which ferments are supposed to find their way into the spinal fluid from the leucocytes. Spät considered the reaction as due to the syphilitic antibodies. Zaloziecki also holds the opinion that the reaction is due to processes analogous to those which occur in the immunity tests; and Weigeldt is firmly convinced that the reaction is conditioned by a pathological alteration of the proteins, which is founded on a greater lability, and a coarser dispersity, of their colloidal state, and is probably identical in the Wassermann reaction and in the flake precipitation reactions. More recent investigations by H. Fischer appear, on the other hand, to support Lange's original view. Fischer was able to show that the albumins behave as protective colloids, and that the globulins have a different precipitating effect which varies in strength in the four different fractions. Lange has recently expressed the decided opinion that the gold reaction represents a quantitative and qualitative protein reaction in the sense that the intensity of the reaction, recognizable in the maximum precipitation attained, is proportional to the absolute protein content; while the position of the precipitation maximum is determined by the protein quotient in such a manner that with a relatively higher globulin content the maximum precipitation occurs in the lower dilutions, and with a comparatively high albumin content, as found in increased permeability, the maximum occurs in the higher dilutions.

Owing to the difficulty of preparing a gold sol of invariably good quality, other methods of investigating the spinal fluid have been suggested in which other colloids are employed which are easier to prepare. But these reactions are to some extent less sensitive and less constant, and the results are more difficult to read off; while some of them are purely quantitative, and fail to express the qualitative reaction-type in the form of a curve as in the gold reaction.

Emanuel suggested the employment of mastic in the place of the gold sol, in which method the starting point for preparing the reagent is a preservable stock solution of 10 per cent. mastic in absolute alcohol. The stock solution should be placed in a refrigerator for two hours previous to further use for the purpose of allowing it to mature and to form a sediment (Kafka). According to Emanuel the solution for performing the reaction is prepared by mixing 9 c.c. of absolute alcohol with 1 c.c. of the stock solution of mastic, then quickly blowing in 40 c.c. of distilled water, which results in the formation of a sol. Eskuchen recommends "fractional" emulsification by the slow and dropwise addition of the water—or conversely by adding the alcoholic solution to the water—which he based on researches of Sachs, who found that when the water was blown too suddenly into the mastic solution the sols which were formed were too sensitive to salt; the more turbid sols, which are also less sensitive to salt, that result from the slow addition of water are said to give serviceable results. With the object of producing uniform sols, Kafka also adds the mastic solution drop by drop to the water while shaking gently, in such a manner that the whole process takes fifty seconds as measured with a stop-watch. Stern and Poensgen controvert the view that the rapidity of the emulsification has necessarily any influence on the sensitiveness of the reagent to salt; and they recommend quickly blowing in. Schönfeld places the mastic solution

in a wide test-tube, or a cylindrical glass measure, or an Erlenmeyer flask, then submerges water slowly beneath it without employing any pressure, then, after closing the vessel at the top, inverts it slowly two or three times. Before employing it for investigation purposes it should be allowed to mature by standing for half-an-hour ("maturing-time" according to Jacobsthal and Kafka), by which the sensitiveness to salt is somewhat lessened.

In his plan of investigation, Emanuel worked exclusively with a sodium chloride concentration of 1.25 per cent. Commencing with a 4-fold dilution of spinal fluid (0.5 spinal fluid + 1.5 salt solution) he prepared a geometrical series of four tubes in the manner already described for the gold test, viz., by transferring from each tube 1 c.c. of liquid into the next tube, thus making a series of 4-, 8-, 16-, and 32-fold dilutions of spinal fluid. A fifth tube containing only 1 c.c. of the saline solution serves as a control. To each of these tubes 1 c.c. of the mastic sol is added, the tubes are shaken, and at the end of twelve hours the results are read off.

In Emanuel's mastic reaction the normal spinal fluid fulfils the part of a protective colloid, i.e., it prevents the precipitation of the mastic, which with sodium chloride solution alone would, and in the control tube actually does, take place; but it is absent in the tubes containing normal spinal fluid. Pathological spinal fluid cancels this protective effect, and according to the degree of reaction precipitation takes place, rising from weaker to stronger reactions, in the fourth, or third and fourth, or in the first, third and fourth, or in all four tubes.

In analogy with the gold reaction, Jacobsthal and Kafka have lengthened the series and have recommended as in their original, but now abandoned, modification of the test, a preliminary trial for the determination of the sensitiveness to salt of the colloid. This is performed by adding to a series of solutions of sodium chloride of 1 c.c. each, and of increasing concentration (0.5 per cent.,

0.6 per cent., 0.7 per cent., 0.8 per cent., 0.9 per cent., 1 per cent., 1.25 per cent., and 1.5 per cent.), the same volume of the reagent, shaking the tubes, and observing the changes which soon appear. In their original modification, Jacobsthal and Kafka employed for the reaction the first dilution of sodium chloride solution which precipitated the mastic sol.

The latest modification which Kafka has introduced to the mastic reaction consists in the addition to the sol of a dye, and in the employment of "normosal" in solution (Sächsische Serumwerke, Dresden) in the place of sodium chloride solution, by which the precipitation of the sol in the high dilutions of normal fluid is avoided. Kafka gives the following directions for this "coloured normomastic reaction." Prepare a concentrated solution of Sudan III in absolute alcohol. Take 0.5 c.c. of this solution and add it to 8.5 c.c. of absolute alcohol. To the 9 c.c. is now added 1 c.c. of the stock mastic solution, and the 10 c.c. are drawn into a pipette and allowed to fall dropwise within fifty to sixty seconds by the stop-watch into a vessel containing 40 c.c. of doubly distilled water. After having allowed this to mature for half to one hour, the investigation of spinal fluid may be proceeded with. Following Goebel's suggestion, Kafka extended the reaction to the left by taking undiluted spinal fluid, dilution 1 : 1, dilutions 3 : 4 and 1 : 2.

More recently, Eskuchen has rightly pointed out the necessity of bringing the mastic reaction under uniform rules. He considers the dilutions 1 : 1 and 3 : 4 may be dispensed with, though with a sufficiency of spinal fluid they are advisable. He stipulates, however, that the dilution 3 : 4, in conformity with its position outside the geometrical series, should be recorded in the curve with only half the usual distance from the adjoining tubes; also dilutions up to 1 : 1000; further, five degrees of precipitation denoted by figures, of which each has two sub-degrees (*a* and *b*, written underneath the figures), viz., less (1) or greater (2) turbidity, transition

from turbidity to precipitation (3), incomplete (4), and complete precipitation (5); moreover, dilution with normosal. As this must only be employed when the mastic sol precipitates with a sodium chloride solution of 0.6 per cent. to 0.8 per cent. (Kafka), every mastic sol should be eliminated which already gives a precipitate in the preliminary trial with 0.5 per cent., but not yet with 0.8 per cent. sodium chloride solution. (Instead of "normosal" it is possible to use a 0.8 per cent. sodium chloride solution with an addition of 1 c.c. of a 0.5 per cent. solution of potassium carbonate to 99 c.c. of water); working with half amounts, i.e., in each tube 0.5 c.c. spinal fluid dilution and 0.5 c.c. mastic sol. After adding the mastic sol, shake the tubes four times, and allow to stand twenty-four hours.

When carried out in the above manner, the mastic reaction gives similar curves to those obtained in the gold reaction, though in typical cases they are not of equal breadth and equal intensity. Thus, e.g., maximum precipitations in the dilutions 1 : 2 to 1 : 16 in paresis, less precipitation in tabes and cerebro-spinal syphilis, indentation at 1 : 4 and 1 : 8 in syphilis, right displacements in meningitis, in pronounced cases up to maximum precipitation in dilutions 1 : 32 and 1 : 64; syphilitic types occur, however, in meningitis, and indeterminate precipitations are not infrequently observed.

As to the clinical working value of the reaction, I am not able to fall in with Eskuchen's view that it should be installed by the side of the gold reaction on the ground that the two reactions most satisfactorily support each other. On the contrary, I consider it unnecessary by the side of the superior gold reaction,¹ and regard the

¹ Lange considers that the reason for this is in the circumstance that, in contrast with other colloids, the gold sol is "hydrophobic" (non-swelling), and relatively homodisperse—the heterodisperse colloids exhibit all degrees between true solutions and coarse suspensions, and have therefore no fixed maximum of precipitation; also that no other colloid exhibits the same degree of sensitiveness with regard to colour changes as the gold sol.

method only as a serviceable substitute in cases in which the gold reaction is impracticable for exterior reasons. I believe that this may also be gathered from Eskuchen's own remarks. I fail to see that the performance of the mastic reaction is simpler than the gold reaction; it is even more complicated owing to the preliminary trial. Only the preparation of the reagent is simpler, which is an advantage that is lost if the gold reaction is to be performed in any case. But according to Eskuchen's own remarks, the gold reaction is superior to the mastic reaction in differential value, and its findings are decisive when both tests diverge. The only value of the mastic reaction, according to Eskuchen, is that its negative outcome renders the performance of the gold reaction superfluous—an insufficient justification for performing it regularly beside the gold reaction, the negative outcome of which, of course, has the same meaning.

A number of other colloid reactions have not proved successful; others have not been sufficiently tested. Among those which have been suggested are: Prussian blue, benzoin resin, Congo red, colloidal carbon, tincture of guaiacum, &c.; and more recently colloidal silver in the form of collargol (Heyden), a sol which is protected by hydrophile colloids, thus enabling a uniform colloidal solution to be prepared. The desire for a constant reagent, simple to prepare, is thus for the above reasons explicable enough, to which may be added that the gold preparations in recent years are often of inferior quality. The substitutes for the gold reaction, however, are by no means equal to it in value, and the newer, noteworthy efforts of Schmitt with the collargol method are unable to alter this fact.

CHAPTER XVII.

THE WASSERMANN REACTION.

IN working out the reaction known by this name, Wassermann, Neisser and Bruck (1906) set out from the idea underlying the method of the deviation of complement introduced by Bordet and Gengou in 1901. This is based on the fact that by the combined action of an antigen and its specific antibody a certain amount of complement or alexin is rendered ineffective, becomes inactive. By the term antigen is meant albuminous matter foreign to the species ("artfremd") e.g., bacteria, foreign blood corpuscles, &c., and antibody is the specific substance provoked in the body by way of reaction to the antigen, whether this has entered the body by infection or by an injection. The complement is a substance present in fresh, so-called active serum, and may be decomposed by heating for some time at 56° C.—it is thermolabile.

If an antigen and complement are mixed with a liquid thought to contain the antibody specific to the antigen, a conclusion can be formed as to whether the investigated liquid contains the antibody by observing whether or not complement has disappeared. The anticomplementary action of antigen and antibody is evidenced by adding a known mixture of antigen and antibody to a mixture of the three substances named which have acted together for a sufficient length of time. The antigen employed consists of red corpuscles of the sheep, and the antibody is the heated, i.e., complement-free, serum of an animal previously treated with this blood (known as anti-erythrocyte serum, amboceptor, preparin, or

hæmolysin; it is not decomposed by heating at 56° C. for half an hour—it is thermostabile).

This mixture of antigen and antibody is so arranged that in the presence of sufficient complement the red corpuscles which it contains are completely dissolved, which is recognizable by the decolorization. Should after a certain time, therefore, the red corpuscles be dissolved, or hæmolysed, this can be taken to prove that the complement present in the first phase after the substances have been mixed has remained free, i.e., that the liquid to be examined, such as a serum, contains no antibody corresponding to the antigen which has been added to it, e.g., a suspension of cholera vibrios. If, on the other hand, the red corpuscles have not been hæmolysed, or only partly so, i.e., complete or incomplete inhibition, the latter in numerous degrees, such as : almost complete, distinct, slight, very slight, trace, &c., of inhibition, then this proves that the mixture no longer contained an amount of complement at its disposal sufficient for the hæmolysis of the red corpuscles, and that, therefore, in the first phase of the reaction, complement had become inactivated, anchored, deviated, which indicates that antibody was contained in the liquid examined for its presence.

Starting from this train of thought, Wassermann, Neisser and Bruck added to a blood serum to be examined for the presence of syphilis antibody a syphilitic antigen consisting of an aqueous extract of syphilitic foetal liver; to this was added complement and the mixture allowed to stand, after which were added sheep corpuscles and inactivated (heated) blood serum of a rabbit that had been previously treated with sheep blood. Hæmolysis was then taken to indicate the absence, and inhibition the presence, of specific, i.e., syphilis antibodies in the serum under examination. Subsequent researches have shown that in this reaction it is not a question of proving the presence of specific syphilis

antibodies, for the result is exactly the same if an extract from normal organs is employed instead of a syphilitic antigen. This, however, has not detracted from the proved great practical value of the reaction. (Non-syphilitic extracts are now employed by most workers, most frequently alcoholic extracts of human or guinea-pig heart-tissue.)

It is not intended to describe here the detailed technique of the Wassermann reaction, and I forgo the full directions for preparing the different reagents. The Wassermann reaction can only be performed with suitable laboratory equipment; but I consider it advantageous that the practitioner should form a clear idea of the different factors that govern the reaction, as only thus can he form a critical opinion on the laboratory findings submitted to him.

In interpreting the Wassermann reaction it is above all necessary to keep in view the fact that it is the result of the combined action of different substances in definite volumetric proportions, which may by no means be arbitrarily changed. It is not a question of processes being disclosed by the reaction, by which the syphilitic body-fluid is formally distinguished from the normal body-fluid. (This holds good at least for serum, but the following remarks, though chiefly based on the better known relations of serum, are of value for the understanding of the Wassermann reaction in the spinal fluid.) It is rather that, with reference to the anticomplementary effect, there exist gliding transitions from perfectly normal serum to those syphilitic sera which are more strongly positive; and there occur in some diseases, moreover, changes in the serum, e.g., in tuberculosis, cancer cachexia, which can effect just as much deviation of complement as is the case with slight changes of the serum in syphilis. Thus, by bringing suitable changes into the order of investigation, it is possible to effect a displacement of the results in opposite directions; on the

one side, by creating such conditions that the reaction will render manifest the fixation of very small amounts of complement, by which in syphilis the outcome is positive with especial frequency, by which, however, there is also an increasing tendency to non-specific reactions (in the case of non-syphilitics); on the other hand, by arranging the reaction in such a manner that it expresses only very strong degrees of anticomplementary action, say, e.g., by the employment of a considerable excess of complement, by which, indeed, a positive reaction is only obtained in indubitably syphilitic cases, while syphilitic cases which only show a small deviation of complement give a negative result. To arrange the reaction so that all cases of syphilis are positive, and all non-syphilitic negative, is an unattainable ideal, and all modifications of the reaction can but strive for this ideal as closely as possible, i.e., to create such conditions that on one hand no non-specific reactions occur, and on the other, that a positive result is obtained in the greatest possible number of syphilitic cases. Theoretical considerations can never provide a standard of measure for the performances of a particular modification; this can only be obtained by practical clinical experience.

In the original Wassermann reaction the prescribed volume of each reagent was augmented to 1 c.c. with physiological saline solution, so that the volume of the liquids acting together in the first phase amounted to 3 c.c., in the second phase to 2 c.c., and the total volume employed in the reaction, therefore, was 5 c.c. Workers now almost exclusively employ half amounts, and some only quarter amounts, while the findings are expressed in amounts of the examined body-fluid which would correspond to the reaction if whole amounts had been employed. Thus, if in working with half amounts a positive Wassermann reaction has been found in 0.25 of spinal fluid, it is customary to say: Wassermann reaction with 0.5 positive.

The volume of the reagents employed in the original Wassermann reaction, in which for the present purpose only the cerebro-spinal fluid comes into consideration, is as follows:—

- (1) Spinal fluid 0.2 c.c. + 0.8 c.c. saline solution.
- (2) Antigen, or extract, about 0.2 c.c. augmented to 1 c.c. with saline solution.
- (3) Complement: 0.1 c.c. + 0.9 c.c. saline solution. (i.e., 1 c.c. of a 10-fold diluted complement = 10 per cent. complement.)
- (4) Sheep corpuscles: 1 c.c. of a 5 per cent. suspension.
- (5) 1 c.c. amboceptor or hæmolytic rabbit serum in a definite dilution.

(1) The spinal fluid is employed by some writers in an "inactivated," by others in an active condition; some inactivate it, i.e., heat it for half an hour at 56° C., only when it contains a large amount of complement, as in meningitis, rare cases of paralysis; or when a considerable admixture of blood is present—in the latter case the spinal fluid must be centrifuged before performing the reaction. Schönfeld warns against carrying out the reaction with spinal fluid containing blood. Lange points out that a scarcely appreciable admixture of blood might be misleading; in aortic aneurism, e.g., complete inhibition occurs with a serum dilution of 1:300; and in such a case an admixture of $\frac{1}{300}$ c.c. (if active spinal fluid is used, which reacts one and a half times as strongly, with $\frac{1}{500}$) plasma to 1 c.c. of spinal fluid could be productive of a positive reaction. More recently the requirement has been more and more put forward to perform the Wassermann reaction simultaneously with active and inactivated spinal fluid. Rizzo first called attention, on the strength of his own observations, and with reference to Busila's experiments, to the importance of this parallel investigation—Eicke's attention was also drawn independently to this fact—and similar observations have subsequently been made by several

writers. The result of inactivation is of a double character, both differing in principle. It effects to some extent the disappearance of the (in general very rare) non-specific inhibitions, e.g., in some cases of meningitis, isolated cases of tumours of the central nervous system; it is also able to lessen the specific inhibitions of syphilis, or even entirely to destroy them; and in progressive paralysis, excepting cases which show a negative serum reaction, the reagines are usually unchanged by heating, or very little, while in cerebral syphilis and in tabes they are more weakened, and in non-nervous syphilis most of all.¹ For this reason, as non-specific inhibitions only very rarely occur, the spinal fluid must be investigated in the active, and also whenever possible in the inactivated, state.

Hauptmann's method, which is an extension of the original reaction of Wassermann-Plaut, is now almost universally employed for the spinal fluid. It consists in performing the reaction with increasing volumes of spinal fluid graded up to 1 c.c., i.e., not only with 0.2 c.c. (20 per cent.), but also, e.g., with 0.4, 0.6, 0.8 and 1.00 c.c. (40 to 100 per cent.) ("Auswertungsmethode.") Lange even augments the amount of spinal fluid to 190 per cent., by diluting the complement with spinal fluid instead of saline solution; for the reaction does not permit of a further increase of volume. Many workers estimate from smaller quantities, e.g., 0.1, 0.08, 0.05, 0.03, and 0.01 c.c.

¹ Rizzo explains that his own differentiation between thermolabile and thermostabile reagines, which he had himself retained for convenience' sake, is incorrect, and that in the process described it is rather a question of the total colloidal state being differently subject to the influence of heating. Two experiments which he performed speak in favour of this view, in which sera that were + + + + in an active state, after inactivating for ten minutes became negative, while after inactivating for half an hour they were + + + +; likewise Renaux's researches, who found in a number of cases that inactivated Wassermann-positive sera after standing for some time at room temperature lost their fixation capacity, though by reheating to 56° C. they regained it.

(2) The antigen employed is usually an alcoholic extract of heart tissue, e.g., 1 grm. of human heart tissue in 10 c.c. of alcohol. Extracts must only be prepared in larger institutions and must be tested for their efficacy and for the optimum amount suitable for the reaction—the so-called “dose.” In suitable extracts this dose is usually a 20 per cent. dilution, i.e., a mixture of two parts extract with eight parts physiological saline solution. Much weaker extracts, i.e., such that show in a reaction performed with blood-serum the necessity for an essentially stronger concentration, e.g., one of 30 per cent. (i.e., three parts extract to seven parts saline solution), are valueless.¹ The extract itself possesses a certain anticomplementary action, which is of importance in determining the dose to be employed. The dose must therefore be chosen so that in the order of experiment no auto-inhibition of the extract arises (more correctly, that the ever-present auto-inhibition should not exercise a disturbing effect on the investigation), which may be excluded by performing collaterally with each investigation a so-called extract or antigen test. The extract is often subject to alteration during the first months after its preparation, so that an accurate determination of the dose is only to be made after a considerable period of time. The extract must only be diluted in a perfectly definite manner, slowly, or rapidly dropping into the saline solution, according to the directions prescribed for each extract by the testing station.

(3) The complement exclusively employed is guinea-pig serum, which is obtained from blood collected from the carotid, or more economically by aspiration from the punctured heart, or by means of a suction appliance on the congested ear, and it must invariably be fresh when employed—best within six to eight hours

¹ Lange recommends for spinal fluid the employment of larger doses of extract, e.g., a 30 per cent. concentration, if the ordinary dose for serum consists of about 18 per cent.

after removal. If the complement is not quite fresh when employed it exhibits considerable variations in strength, whereby its hæmolytic power, i.e., its dissolving effect, and its anchorage, or deviation capacity, do not necessarily run parallel. (Lange asserts, indeed, that fresh serum taken from healthy guinea-pigs is quite constant, and that he has never observed any differences in anchoring capacity or "deviability.") It is therefore now customary to determine its hæmolytic power by a preliminary test. A complement of more equal power may be obtained by mixing together the serum from several healthy, well-nourished guinea-pigs.

(4) The sheep blood is collected from the jugular vein in a vessel containing glass beads which is shaken during the process. It may be used some time if suitably preserved. For the reaction a 5 per cent. emulsion of the blood corpuscles—obtained by centrifuging and repeatedly washing with saline solution—is employed, i.e., 1 c.c. of blood corpuscles to 19 c.c. of sodium chloride solution; it is also possible to use instead an 11 per cent. emulsion of the total blood. (Some writers consciously, others unconsciously, employ a 5 per cent. emulsion of the total blood, in which case the conditions of investigation must be modified.) If the centrifuged sediment is to be employed, the blood must be centrifuged in a very good machine and the washings carefully drawn off with a capillary tube, otherwise too weak an emulsion results. The blood corpuscles are washed by adding physiological saline solution after removal of the plasma, again centrifuging and removing the salt solution; this must be repeated several times. When employing the total blood, which is more convenient, 11 c.c. are distributed in a few centrifuge tubes, washed three times with optional amounts of saline solution, carefully collecting the washings and rinsing the remainder of the corpuscles into a vessel, which is then filled up to 100 c.c.

(5) The amboceptor employed is the blood-serum of a

rabbit which has been previously treated by repeated intravenous or intraperitoneal injections of 50 to 100 per cent. sheep blood. The amboceptor must be inactivated previous to use, i.e., it must be heated for some time at 56°C .; it can be kept. A good amboceptor must be able to hæmolyse with at least a 1500th to 2000th dilution, i.e., with this dilution 1 c.c. must be capable of completely dissolving 1 c.c. of a 5 per cent. emulsion of sheep blood in the presence of 1 c.c. of 10 per cent. complement. The effectiveness of the amboceptor is tested by titration of a graded series of dilutions, e.g., 1 : 100, 1 : 500, 1 : 1000, 1 : 3000, 1 : 4000. To each 1 c.c. of these dilutions is now added 1 c.c. of a 5 per cent. emulsion of sheep blood and then a ten-fold diluted complement; the tubes containing these amounts of liquid are placed for half an hour at incubator temperature and readings taken in which dilution complete hæmolysis has been brought about and in which this has no longer been the case; after this, a further series of dilutions is prepared which lie between the two values, until the hæmolysis titre has been exactly determined. The four-fold hæmolytic dose of amboceptor is at present usually employed for the Wassermann reaction, or, as it is usually expressed, four units of amboceptor or antibody, e.g., if the amboceptor under the given conditions just hæmolyses in a dilution of 1 : 3200, then an 800th dilution. Amboceptor and blood are added together, after equal quantities have been mixed and incubated for half an hour—the blood is thus “sensitized.” (Some writers recommend the mixture to be kept at room temperature, others even direct it to be placed on ice.)

If half amounts are taken, the reaction, preceded by a hæmolytic test, is performed as follows: In estimating the value of the complement in the preliminary test, the fact is proceeded from that in order to effect the hæmolysis of 1 c.c. of a 5 per cent. emulsion of sheep blood, and employing four hæmolytic units of ambo-

ceptor (this latter evaluated with 10 per cent. complement), about 2·5 per cent. complement is required (i.e., 0·025 undiluted complement brought up to 1 c.c. with sodium chloride solution). This volume of complement thus constitutes the approximate mean value of the preliminary test series. If for the preliminary test a fifty-fold complement dilution is prepared (one part complement and four parts salt solution—of this mixture one part to nine parts of saline solution), then 0·1 c.c. of this dilution constitutes a 0·2 per cent., and working with half amounts, a 0·4 per cent. amount of complement; 0·4 c.c., therefore, a 1·6 per cent. amount of complement, and so on. The following test series is thus prepared:—

50-fold dilution of complement		Physiol. sal. solution		Mixture of blood and amboceptor ¹
0·9	...	0·6	...	1·0
0·8	...	0·7	...	1·0
0·7	...	0·8	...	1·0
0·6	...	0·9	...	1·0
0·5	...	1·0	...	1·0
0·4	...	1·1	...	1·0

¹ 0·5 each of the 5 per cent. emulsion of sheep corpuscles and the 4-fold hæmolytic dose of amboceptor, sensitized.

The whole series is placed for half an hour in the incubator at 37° C., or in a water bath at 40° C., and then stood for an hour at room temperature; the result of the preliminary hæmolytic test is now read off, viz., the smallest amount of complement which has brought about complete hæmolysis of the blood.

As the unit of complement determined in the preliminary test is necessary for the solution of the red corpuscles, without the addition of antigen and spinal fluid, and as, moreover, the antigen alone anchors about $1\frac{1}{3}$ units of complement, and a slight excess must be calculated for the auto-inhibition of the spinal fluid—generally but very slight, however—I therefore employ for the main test $2\frac{1}{2}$ hæmolytic units of complement. If thus in the preliminary test a dose of 0·7 c.c., i.e., a 2·8 per cent. amount of complement, has brought about

hæmolysis, then a 7 per cent. dilution of complement is prepared ($2\frac{1}{2} \times 2.8$) by mixing 0.7 c.c. of complement with 9.3 c.c. of physiological saline solution (or correspondingly larger amounts in the same proportions according to the number of specimens of spinal fluid to be investigated). This dilution can then be mixed in equal parts with the antigen, and the two reagents may be added jointly in the main test, not forgetting, however, to set aside a small amount of the dilution of complement which is required for the spinal fluid control.

The first phase of the chief test is now arranged, e.g., in the following manner :—

Spinal fluid		Physiol. sal. solution ¹		Mixture of com- plement and antigen ²		Complement
0.015 ³	...	0.485	...	1.0	...	—
0.025	...	0.475	...	1.0	...	—
0.05	...	0.45	...	1.0	...	—
0.1 ⁴	...	0.4	...	1.0	...	—
0.25 ⁵	...	0.25	...	1.0	...	—
0.4	...	0.1	...	1.0	...	—
0.5	...	—	...	1.0	...	—
0.5	...	0.5	...	—	...	0.5 ⁶
	...	0.5	...	1.0	...	—

¹ The physiological saline solution increases the volume of spinal fluid to 0.5 c.c.

² Equal parts of antigen and $2\frac{1}{2}$ hæmolytic units of complement.

³ This tube can be omitted, or instead of the first two tubes a single tube with 0.02 spinal fluid + 0.48 saline solution can be employed.

⁴ Amount of spinal fluid according to the original method (0.2 c.c. if whole amounts are employed). The following tubes are set up according to Hauptmann's method.

⁵ If there is a deficiency of spinal fluid this tube and the next can be replaced by one tube containing 0.3 + salt solution 0.2.

⁶ In this series the mixture of complement and antigen is left out, and 0.5 of $2\frac{1}{2} \times$ hæmolysing complement employed instead.

The tubes must be well shaken and allowed to stand for three-quarters to one hour at incubator temperature. Then to each tube is added 1 c.c. of the mixture of blood corpuscles and antibody. (This is the second phase of the chief test.) After having remained one hour in the water-bath or the inspissator the results are read off. The control tests for auto-inhibition in the last two

tubes (spinal fluid and antigen tests) must show dissolution, or the investigation is valueless.¹ The reaction performed in the manner described necessitates 1·47 to 1·84, or in round figures 1·5 to 2 c.c. of spinal fluid; with simultaneous employment of active and inactivated fluid double the amount is required.²

Most of the numerous modifications and "improvements" of the Wassermann reaction are not applicable to the spinal fluid. Hauptmann's method with increasing volumes of spinal fluid has been referred to as a useful extension of the reaction; likewise the method of estimating in a positive reaction from smaller amounts of spinal fluid than those originally prescribed (0·2 c.c.) and based on the circumstance that the stronger the reaction the less amount of spinal fluid is needed to show a positive result. In a similar manner the strength of the reaction can be determined by grading the amounts of complement (Klieneberger, Kaup)—the reaction is the stronger positive the larger the doses of complement deviated. Information as to the degree of the reaction can also be obtained by varying the doses of extract; thus with smaller amounts of extract stronger reactions are expressed. Acting on this principle some writers work with two different antigen doses; and some consider it of importance to perform the reaction simultaneously with two different doses of extract; and Sormani finds his estimation of the reaction on the gradings of the extract, and attempts to express its degree numerically by the ΣI = the syphilis index. Sormani avoids on principle all excess of complement, determining by a preliminary test the precise amount of complement that just effects hæmolysis in the presence of a definite

¹ Lange requires for the auto-inhibition test that any excess of complement must be avoided, i.e., the unit of complement must be employed, corresponding to about 3 per cent. complement.

² Lange, in order to economize spinal fluid, not unjustifiably recommends the employment of only the maximum dose when the protein content of the spinal fluid is below 1 per thousand.

antigen dose, and employing the amount of complement thus found for the chief investigation.

The essential nature of the Wassermann reaction has not hitherto been adequately explained. The reaction in serum seems most explicable on the assumption of a globulin alteration in consequence of which a precipitation of globulin results from the interaction of syphilitic serum with an organic extract. Now, in the Wassermann reaction, in contrast to the flake precipitation reactions, this precipitation does not really occur, it is only attained *in statu nascendi*, as Sachs expresses it, for the fine degree of increase of the disperse particles, which fails to lead to a visible precipitation, is already sufficient to bring about an anticomplementary effect.

In the case of the Wassermann reaction in spinal fluid, which was first demonstrated by Wassermann and Plaut in 1906, the conditions are essentially more complicated. Here the question arises whether the reagines originate locally, or whether they reach the spinal fluid from the blood. Zaloziecki has proved the latter to be the case in syphilitics with non-meningitic diseases, and his findings have been confirmed by other writers.¹ Zaloziecki also plausibly points out that some of the reasons brought forward in favour of a local origin of the reagines need further investigation, as the different colloido-chemical constitution of serum and spinal fluid might possibly account for certain differences in the degree of the reaction. The occasional occurrence, for

¹ Observation of cases of non-syphilitic meningitis have multiplied of late in which the spinal fluid has shown a positive Wassermann reaction, also in non-syphilitics with a negative serum reaction. The explanation is perhaps to be found in the fact discovered by me that liquids in which larger quantities of colourless blood corpuscles have become disintegrated exhibit anticomplementary characters. When recording these researches I pointed out the necessity for extended after-examinations in cases of meningitis. In some cases of tumour of the central nervous system, however, a non-specific positive Wassermann reaction was found in the absence of any increase of cells worth mentioning. (See also p. 159.)

instance, of a negative Wassermann reaction in the spinal fluid with a positive serum reaction, or of a stronger positive reaction in the spinal fluid than in the serum, might be attributable to a specially favourable proportion in the fluid between the substances promoting the reaction and those inhibiting it. (On the other hand, Kafka sought to prove experimentally that the conditions for the reaction in the spinal fluid are even less favourable than in the serum.) Notwithstanding these objections, however, which are more of a theoretical nature, Zaloziecki has not excluded the possibility of a local origin of the reagents which give the Wassermann reaction in the spinal fluid, and this seems now to be the generally accepted view.¹

There is by no means a consensus of opinion, however, on the nature of their origin. Wassermann and Lange have sought to prove that the reagents are derived from the disintegration of the syphilitic's lymphocytes, which contain a specific proportion of them, thus reaching the spinal fluid; but these researches have not been verified.² Other writers imagine that the anticomplementary effect of the syphilitic spinal fluid owes its origin to the cleavage products (amino-acid, lipase), which reach the fluid from parenchymatous organs subject to pathological destruction. Weil and Braun suppose that the reaction is due to local products similar to antibodies which are developed by way of answer to the passage of specific products of the body cells ("körpereigene spezi-

¹ R. Bauer, however, still holds that the positive Wassermann reaction of the spinal fluid originates in the serum, i.e., it is "as it were, a copy of it" ("ein Abklatsch derselben"). (In discussion—*Jahrbuch f. Psych.*, 41, 1922, p. 279.)

² Lange, however, still continues to uphold the view that the syphilitic reagent only originates from infiltration of the round cells of the meninges. He thinks that the Wassermann reaction is essentially and absolutely identical with the gold reaction, i.e., it is nothing more than a qualitative and a quantitative protein reaction; the protein of the syphilitic spinal fluid, however, originates by autolysis of the round cells of the fluid.

fischer Zellbestandteile"). Kafka believes that the reagents of the spinal fluid owe their existence to the specific action of spirochaetes on the tissues, and that therefore a positive Wassermann reaction in the spinal fluid affords evidence of the presence of spirochaetæ, or the specific substances of which they bring about the formation.¹

With regard to the relationship between the Wassermann reaction and other pathological changes of the spinal fluid (increase of cells, increase of albumin, gold reaction) it has not been possible to prove any certain parallelism in spite of numerous researches²; it is only possible to say that the Wassermann reaction in the spinal fluid is almost invariably limited to such cases in which other pathological findings are present.

Besides syphilis and a few isolated cases of meningitis, a positive Wassermann reaction of the spinal fluid is only met with in diseases which only rarely come under our notice, such as sleeping-sickness and leprosy. This enables us to say with sufficient certainty for practical purposes that a positive result—technically free from all objection, of course—proves the presence of syphilis; but only of a meningitic syphilis, not of a syphilitic affection of the central organ, for the Wassermann reaction also occurs in cases of syphilitic infection in which objective signs of organic nervous diseases are absent, though in such cases in an essentially smaller percentage than in diseases of the central nervous system.

¹ Kafka even thinks that the serum reaction in paralysis is due to the passage of reagents from the spinal fluid into the blood; while Plaut advances the view that in paralysis the nervous system is not the sole source of origin of the reagents, for early stages sometimes show a positive blood reaction when the spinal fluid is still negative, and in remissions the reactions in the spinal fluid can disappear while still remaining positive in the serum; also, finally, treatment influences now one, now the other reaction, independently of each other.

² Lange still asserts that the Wassermann reaction gives perfectly parallel results with the gold reaction (not identical results, for the Wassermann reaction is much coarser), because the quantitative behaviour of both reactions is dependent on the albuminous increase. (Cf. note on previous page.)

A positive Wassermann reaction is to be observed in the spinal fluid in a small percentage (up to 3 per cent.) already in the primary stage of syphilis after the appearance of the serum reaction and glandular swelling. In cases of stage II and in latent syphilis, in which no nervous complications are present, a positive reaction is found in the first years in about 8 to 10 per cent. of cases, and in later years not much more frequently. With reference to the frequency of the Wassermann reaction in congenital syphilis and in gummatous syphilis, the literature of the subject gives contradictory data. In nervous affections the frequency of positive results heightens considerably. The reaction in a large number of early cases of nervous syphilis (neurorelapse, &c.) is positive by Hauptmann's method of estimation with increasing volumes. Also, in cerebro-spinal syphilis and tabes with the employment at first of 0.2 spinal fluid the results are negative in a large number of cases; not until larger volumes are employed is a positive reaction found (tabes in 50 to 80 per cent.; cerebro-spinal syphilis in 60 to 75 per cent.). On the other hand in progressive paresis in the great majority of cases the reaction is positive with 0.2 spinal fluid, and in very many cases with considerably smaller amounts (according to my investigations in collaboration with Volk in about 60 per cent. of the cases with 0.03 c.c. spinal fluid).

The differential value of a positive Wassermann reaction in the spinal fluid is therefore as follows:—

(1) In the presence of an organic nervous lesion a positive Wassermann reaction in the spinal fluid proves its syphilitic foundation, also if the reaction is only positive when larger amounts of fluid are employed—excepting meningitis and the rarer cases of organic non-syphilitic disease, e.g., multiple sclerosis, or a syphilitic person with spinal fluid anomalies or a combination of a syphilitic and a non-syphilitic lesion of the central nervous system.

(2) In suspected progressive paresis a negative Wassermann reaction in the spinal fluid speaks with the greatest probability against the presence of such a lesion.

(3) In case of doubt a very strong positive Wassermann reaction in the spinal fluid increases the probability for diagnosis of progressive paresis.

(4) With collateral investigation of active and in-activated spinal fluid a marked difference in the results of the reaction may be taken to weigh against the diagnosis of paresis.

CHAPTER XVIII.

THE FLAKE PRECIPITATION REACTIONS.

SHORTLY after the Wassermann reaction was discovered researches were begun with the theoretical object of penetrating more deeply into its essential nature. A further, practical, aim was that of obviating the complicated process of the fixation of complement, and of economizing the material necessary for establishing a hæmolytic "system"; and by means of a precipitation method to overcome these disadvantages. Following upon the fundamental researches of Porges and Meier, which led to a precipitation reaction with lecithin, a succession of other methods were suggested, among which the reaction of Hermann-Perutz (sodium glycocholate and cholesterin) conquered a certain position. But it was soon evident that all these reactions were insufficiently sensitive or not specific enough. Not until recent years have methods been found which in regard to their practical results are almost equivalent to the Wassermann reaction, and are perhaps destined ultimately to supplant it. As the methods are comparatively simple—though they demand exact work and a certain experience—their employment by the side of the Wassermann reaction may already be worth recommending. The results which the reactions give with spinal fluid, however, are not as yet so satisfactory as with blood-serum.

Two reactions are at present competing for supremacy, the Sachs-Georgi test and the so-called third modification of Meinicke's test.

For the stock solution Sachs and Georgi employ a cholesterinized alcoholic extract of human or beef heart

tissue. Its value is determined once for all by adding different amounts of a 1 per cent. alcoholic solution of cholesterin to the concentrated 1 : 5 extract, and also to different degrees of dilution of the extract with alcohol. As a rule the optimum is found in a mixture of 100 c.c. raw extract with 200 c.c. of alcohol and 13.5 c.c. solution of cholesterin. The solution for the investigation is prepared by diluting this stock solution to six times its volume with physiological saline solution. First one part of the saline solution is carefully added and shaken horizontally, then ten minutes later the remaining four parts are added all at once. The diluted extract is opalescent, but clear and translucent. The saline solution (0.85 per cent.) must, of course, be sterile and clear; it should be passed through filter paper before use.

The reaction is performed by taking spinal fluid which has been inactivated for five minutes—Plaut considers the inactivation unnecessary; while Kafka advises it—in decreasing amounts (0.5, 0.25, 0.15, 0.1, and 0.05 c.c.; with a deficiency of fluid only the first and the last are taken, viz., 0.5 and 0.1 c.c.); and mixing 0.25 c.c. of the diluted extract in each case. Control tests of the spinal fluid and the extract are also arranged; for the former with dilute alcohol instead of with extract, and with saline solution of suitable strength for the extract. The mixtures are incubated for eighteen to twenty-four hours; according to Plaut for spinal fluid two hours in the incubator and further standing at room temperature is sufficient, as the non-specific precipitations that occur in the case of serum in these conditions have not been observed in the spinal fluid. The readings should be taken with a magnifier, or still better with Kuhn and Woihte's agglutinoscope, with a pure white light. For the perception of the minutest flakes Plaut recommends the use of the diaphragm with which the apparatus is now provided. He designates a reaction as slightly

positive when flakes are only just perceptible on a bright ground, but are distinctly seen on a dark ground; while if the minutest flakes are only to be seen on the dark ground the reaction is questionably negative, and with a uniform turbidity the reaction is doubtful.

The test is a specific one in the same sense as the Wassermann reaction, i.e., it is only positive in syphilis. (Plaut found a positive serum reaction in a case of meningitis, analogous to the Wassermann reaction.) But according to the researches hitherto made, the reaction fails for the estimation of amounts of spinal fluid up to 0.5 c.c. much more frequently than the Wassermann reaction; though in a recent work by F. K. Georgi, a brother of the late co-founder of the reaction, the employment of 1.5 c.c. of spinal fluid with 0.75 c.c. of diluted extract, and incubation for twenty-four hours, is said to give even better results than the Wassermann reaction. The employment of half amounts in the Sachs-Georgi test appears to be possible without disadvantage.

For Meinicke's third modification test an extract of horse heart is prepared in the following manner: The heart tissue is shredded, dried in the incubator, then pulverized, and shaken for one hour with 9 parts of ether. To the residue after filtering are added 9 parts of 96 per cent. alcohol, and the whole is allowed to stand for a few days at room temperature, shaking frequently. The value of the extract is then estimated by arranging series with diminishing amounts of extract and increasing amounts of alcohol, thus: 0.4 E + 0.1 A, 0.3 E + 0.2 A, 0.25 E + 0.25 A, and so on. To each of these dilutions of 0.5 c.c. is added 0.25 c.c. of distilled water, which is then well mixed and allowed to stand for an hour. The extracts will then become more or less turbid. To each tube is now quickly added 3.5 c.c. of distilled water and the contents again mixed. The right concentration of the extract is in the tube which immediately shows a

distinct turbidity, while retaining its translucency, after the first addition of water; that shows, moreover, a milky opacity during the time of standing for one hour, and which milkiness on the second addition of water, while becoming somewhat clearer, still distinctly remains. Too strong concentrations immediately become opaque after the first addition of water, while too weak concentrations become almost perfectly clear again after the second addition of water.

The optimum dilution thus found is employed as a stock solution. From it the solution for use in the investigation is prepared immediately before it is undertaken. One part of stock solution is mixed with a $\frac{1}{2}$ part of distilled water, and one hour later 7 parts of a 2 per cent. sodium chloride solution are quickly added. The reaction with spinal fluid is performed according to Schmidt and Pott by taking 1 c.c. of inactivated spinal fluid and 0.8 c.c. of the diluted extract. Results are read off after one hour's incubation, during which time the temperature should not fall below 37° C.

The specificity of Meinicke's test is very similar to that of Sachs-Georgi. In 190 out of 201 cases, Schmidt and Pott found the reaction to be in agreement with the Wassermann reaction. In 11 out of 52 cases with a positive Wassermann reaction the Meinicke reaction failed. The writers think, however, that still better results might perhaps be obtained with the employment of stronger extracts and smaller amounts of spinal fluid.

More recent research has tended towards a further simplification of the flake reactions (Hecht, Bruck and others, afterwards joined by Meinicke himself). Among these methods may be mentioned Dold's "turbidity test," which has also been tried for spinal fluid, but has also failed, in common with other flake precipitation tests, more frequently than the Wassermann reaction. Rochow arranged the reaction with increasing amounts of inactivated spinal fluid (5 tubes of 0.2 to 1.0); Kiefer with

only one dose of 1.0 (for the reaction with serum 0.4 is used). A control is set up consisting of 1.0 spinal fluid + 2 drops of formalin solution diluted with physiological saline solution as 1 : 3. Then to each remaining tube is added 1 c.c. of the extract diluted according to the original directions as 1 : 11. (The extract is an alcoholic cholesterinized beef-heart extract, and is to be obtained from the Behring Works, Marburg.) After shaking a little and placing in the incubator for four hours, the results are read off. A second reading after twenty-four hours' standing at room temperature is recommended for spinal fluid. The readings are taken by holding the test-tube stand about two yards (metres) away from a window and looking through the tubes obliquely from below, comparing whether any difference is shown between the formalin control and the reaction tubes. With a negative reaction the liquid is slightly bluish and clear; a positive reaction gives a dull grey or greyish-blue turbidity, while with a strongly positive reaction flakes are seen at the bottom of the tube.

Various opinions exist as to the nature of the flake precipitation reactions. Some writers imagine that the extract colloids disturb the sodium chloride equilibrium of the serum-globulins by depriving them of that salt, which disturbance occurs to a stronger degree in syphilitic body-fluids than in others. Others consider that it is due to a combining reaction between serum-globulin and extract lipoid. Ott and Winkler succeeded in almost every case in sensitizing guinea-pigs against human protein by the injection of the flocculent precipitate originating from a Sachs-Georgi reaction. According to Wassermann's view the flakes contain also human serum-protein. With flakes that had been produced artificially in negative sera it was only possible to produce a much smaller degree of sensitization. The writers think that with a positive result a more stabile specific combination between extract lipoid and serum protein

takes place, and from this they conclude that in the Wassermann reaction a "Wassermann aggregate" is also actually formed. The writers leave the question undiscussed whether it originates from a true reaction between antigen (lipoid) and antibody in Wassermann's sense, or whether it is due to a non-specific colloidal chemical reaction between extract lipoid and serum-globulin which has become unstable; or perhaps to a successive combination of both reactions.

CHAPTER XIX.

THE HÆMOLYSIN TEST.

STIMULATED by similar investigations of the aqueous humour, it occurred to Weil and Kafka to examine whether, and under what circumstances, the amboceptor occurring in normal blood against sheep corpuscles can be shown to exist in the spinal fluid.

With this object in view they worked out the following method: 5 c.c. of clear, colourless spinal fluid free from blood—with admixture of blood only a negative finding may be interpreted—are mixed in a graduated centrifuge tube with 0.5 c.c. of a 5 per cent. emulsion of sheep corpuscles. The mixture is well shaken and placed in the incubator at 37° C. for two hours—or for one and a half hours in a water-bath at 40° C. A tube containing 5 c.c. of physiological saline solution and the same volume of the blood-emulsion serves as a control. While the tubes are in the incubator they must be repeatedly shaken.

Directly following this first phase of the investigation, the object of which is the sensitization of the sheep corpuscles by the antibody should it be present in the spinal fluid, a complement investigation is arranged by mixing graded amounts of 2-fold diluted complement each with $\frac{1}{2}$ c.c. of an emulsion of sheep corpuscles and submitting it to inspissator-warmth. The grading is arranged on the following plan¹:—

¹ In this preliminary test amboceptor is not added. Larger amounts of complement are able to hæmolyse sheep corpuscles alone on account of the normal amount of amboceptor in the serum. The normal amboceptor content can be diminished by adding to each cubic centimetre of fresh complement $\frac{1}{2}$ c.c. of concentrated sheep blood and sharply centrifugating at once (Weil), or by placing the complement in a cold place (Boas and Neve).

2-fold diluted complement		Physiological saline solution		5 per cent. sheep blood
0·4	...	0·1	...	0·5
0·3	...	0·2	...	0·5
0·2	...	0·3	...	0·5
0·1	...	0·4	...	0·5
0·06	...	0·44	...	0·5

After expiry of the prescribed time the centrifuge tubes containing the mixture of spinal fluid and blood emulsion are taken out and centrifuged. If the liquid has turned yellowish—the control must, of course, have remained perfectly colourless—then antibody and complement were present in the spinal fluid, or hæmolysis would not have taken place; if it has remained colourless, it is poured off as completely¹ as possible; the sediment is then augmented to 0·5 c.c. with physiological salt solution (hence the graduated centrifuge tube), and that amount of complement added which in the preliminary test had been found just to dissolve a trace. The dose necessary is usually 0·2; complement which fails to hæmolyse with a dose of 0·4 is not suitable. (Weil and Kafka originally employed 10 c.c. of spinal fluid and 1 c.c. emulsion of sheep corpuscles for their reaction; after centrifuging they did not augment with saline solution to 0·5 c.c., but to 1 c.c.; divided the emulsion into two equal portions and treated one portion with the amount of complement that just showed a trace of hæmolysis, and the second portion with the largest amount of complement which was not yet sufficient to hæmolyse.) The tubes are now shaken, and after three hours in the incubator the reaction can be read off.

Kafka differentiates between six degrees in the positive reaction according to the amount of hæmolysis—complete, strong, moderate, weak, trace, minute trace of hæmolysis—which he designates by the numbers 1 to 6.

¹ The spinal fluid which is poured off can still be employed for the Wassermann reaction.

In order to ascertain to what degree antibody has passed from the serum to the spinal fluid, Kafka tests the antibody-content of the serum in a similar way to that of the spinal fluid. In some cases the investigation of the serum is of importance because in certain conditions the normal amboceptor is not present in the serum or is lessened in amount, and for this reason the reaction can be negative.

A positive hæmolysin reaction is almost invariably found in acute meningitic lesions of a syphilitic as well as a non-syphilitic character, and in these diseases there is usually a passage of normal amboceptor *and* of complement. Among the syphilitic lesions of the central nervous system it is found above all in progressive paralysis, in about 80 per cent. of which cases a passage, usually only of normal amboceptor, seldom of complement as well (about 7 per cent.)—but then almost invariably with a very considerable passage of amboceptor—can be demonstrated. In other cases of nervous syphilis, and also in pupillary disturbances, the reaction is positive, but with less frequency; and it has even been observed in a few cases of syphilis II without nervous symptoms, and in latent syphilis. The degree of passage in general paresis varies between $\frac{1}{200}$ and $\frac{1}{12}$ (Kafka and Rautenberg).

As will be understood from the foregoing remarks, the hæmolysin reaction is not the sign of a change in the blood like the immunity tests, but it indicates, as its discoverer supposed, an alteration of tissues, particularly an increased permeability of the meningeal vessels. In cases in which the condition is uniform and chronic, as in paresis, chronic meningitis is not regarded as wholly accounting for it; it is probably due to “functional” changes in the vessels brought about by syphilis. It is in the deficient regenerative power of these, it is thought, that the essential character of paresis lies, as the same disease of the vessels also affects the cortex, thus leading

to the severe, progressive character of the symptoms. The theory serves to make clear the practical importance which the writers ascribe to their reaction, for from a longer continuance of the same they conclude a prognostically unfavourable lesion of this nature. (A still more indirect application of the theory of meningeal permeability is in Gennerich's supposition that in so-called metasyphilis the disease is due to a progressive permeation of the cortical layers with spinal fluid. This view will not bear criticism.)

In total opposition to Weil and Kafka, Zaloziecki (who found their reaction to occur occasionally also in cases of tumours of the central nervous system, of cerebral abscess, Broadbent's apoplexy, caries of the vertebræ) ascribes no importance to it, as in his view it is strictly dependent on the protein content of the spinal fluid, and must occur in all diseases accompanied by an increase of protein in the fluid.¹ Kafka, on the other hand, submits that in cerebro-spinal syphilis the reaction is generally negative, even with a large albuminous content, while in paresis it is often present with a small protein content, and that it is, moreover, positive in some cases when all other pathological reactions in the spinal fluid are absent, which is also confirmed by Boas and Neve. Kafka also sought to prove, in collaborative research with Rautenberg, that the reaction shows no parallelism with the Nonne-Apelt reaction, and only a slight parallelism with the globulin components precipitable by a 33 per cent. saturation of ammonium sulphate. Kafka points out further that the ratio of antibody-content to the globulin reaction (the "antibody index") is in paresis larger on the average than in other syphilitic forms of disease and than in meningitis.

I do not think that Zaloziecki's wholesale rejection of the theoretical foundation of the reaction is justified;

¹ Similarly Lange considers the reaction an "unnecessary variant of the granulocyte finding."

but I doubt if it has any practical value, with all the theoretical interest it deserves. In any case this would have to be first established by more extensive verification tests, which are not yet forthcoming on account of the large amount of spinal fluid the reaction necessitates. So far it seems to me very questionable that the reaction possesses any differential value in difficult cases; and in cases in which a sufficient result is obtained from the other methods of investigating the fluid it is on practical grounds unnecessary. Also from the prognostic viewpoint I cannot venture, as Kafka does, to draw unfavourable inferences from its occurrence in non-paretic cases.

With regard to meningitis, Salus claims to have often noticed the Weil-Kafka reaction as a first sign; he also sees a favourable prognostic sign in the disappearance of the reaction in meningitis. Soyka also claims to bring the reaction into requisition for the early diagnosis of otogenous meningitis. But in this connection other methods of examination are no doubt superior to it.

A method of proving the presence of complement with small amounts of spinal fluid owes its origin to Salus. This method only comes into consideration for meningitis, as in paresis the complement is usually absent from the spinal fluid. Salus adds to 1.0 c.c. spinal fluid 0.1 c.c. of 10 per cent. emulsion of sheep corpuscles and submits the mixture to inspissator warmth. (Control test with 1.0 physiological salt solution.) If no hæmolysis has occurred, two units of amboceptor corresponding to the hæmolysis of 1 c.c. of sheep-blood are added to each tube and the mixture again left for half an hour at a temperature of 37° to 40°C.

CHAPTER XX.

THE DIAGNOSTIC AND PROGNOSTIC IMPORT
OF LUMBAR PUNCTURE.¹

IN order to gain a clear idea of the diagnostic limits of spinal fluid investigation, it is essential to remember that pathological reactions of the fluid can have a very manifold origin, and can occur in the most varied clinical pictures. Thus, alterations of the fluid are found accompanying infectious meningeal inflammations, whether associated with parenchymatous foci or not, in which meningitic symptoms predominate; while, on the other hand, they can be accompanying symptoms of e.g., arterio-sclerotic encephalomalacia, without arriving at any kind of clinical expression. Lying between these two extremes are multifarious lesions of a general or a local character, which can lead to alterations in the spinal fluid, and can occasion clinical symptoms which indicate diseased conditions of the meninges, or which may also be absent. Alterations in the fluid are thus found to occur as a result of infectious lesions which may have their seat in the central organ or in its neighbourhood without the meninges having been attacked; or also in consequence of chemical, mechanical, or thermal injuries to the central organ and its membranes; and pathological findings of the fluid occur in a number of general infections, in chronic intoxications, disturbances of metabolism, &c. Finally, alterations in the

¹As the differential value of the various reactions has been repeatedly discussed in the foregoing chapters it has been possible to keep the present chapter comparatively short.

fluid also occur as a result of lesions which obstruct the movement of the fluid, and which develop in the section of the spinal fluid cavity below the occlusive lesion.

Now, although certain degrees of the different reactions and certain combinations of several reactions (the "reaction spectrum") show a predilection for certain pathological groups—out of which the diagnostic value of lumbar puncture emerges—yet the association of spinal fluid alterations with particular pathological states is not uniformly determined, for which reason it is generally impossible to form a clinical diagnosis from the spinal fluid findings alone. The results of lumbar puncture can therefore only be interpreted in conjunction with the clinical picture, and by taking all the varied significance of the spinal fluid findings fully into account. By proceeding in this manner lumbar puncture is found to be of pre-eminent value; but to expect it to replace all diagnostic considerations in the clinical field is to misunderstand its foundations.

In addition to this fundamental fact it is also essential to retain a clear view of the varied significance of the different reactions.¹ A positive Wassermann reaction in the spinal fluid can be regarded for all practical purposes as adequate proof of the presence of a syphilitic infection; also the degree of the reaction can be guardedly brought into requisition, having regard to the clinical picture, for the diagnosis of the particular variety of the disease (cf. Chapter XVII). An increase of cells in the fluid indicates the presence of inflammatory meningeal changes,² and certain conclusions may be drawn from

¹ As the border between normal and pathological is not uniformly laid down, it is necessary in statistical elaboration of the lumbar puncture findings not only to give precision to one's own point of view, but also to include such cases, separately for the different reactions, which belong to contested borderland regions. Only in this way can discordances be eliminated which arise from dissimilar interpretation of the results.

² In the discussion of the changes in the fluid in early syphilis the view has been stated (Merzbacher, Nissl) that pleocytosis is not

the appearance of the cells as to the extent of these lesions and whether they are recent or not (cf. Chapter X). The increase of globulin and albumin can originate in two different ways that should be kept apart in principle, though in practice they often work together. It can be due to an increased production of "endogenous" protein (Lange)¹, or to a passage of protein from the blood ("exogenous" protein, Lange). The first mode of origin, which occurs with especial frequency in chronic lesions, e.g., in syphilis, exhibits a conformity to law ("Gesetzmässigkeit") which distinctly emerges from the spinal fluid changes in syphilis. It makes its appearance later than the increase of cells, but in a not inconsiderable number of cases it remains after the cell-increase has disappeared. It would thus appear to be the expression of a more advanced phase of disease, and often of a permanently remaining anomaly after the abatement or after the disappearance of the local injury. This would also explain the fact that a slight, isolated protein increase is not infrequently met with in some chronic diseases of the central nervous system, particularly in multiple sclerosis. A derivation of protein from the blood occurs on the one hand in hæmorrhages in the subarachnoid space, and on the other in the passage of blood-plasma into the spinal fluid, whether this be due

necessarily ascribable to meningitis, i.e., that it need not be due to anatomical changes in the meninges. As regards chronic forms of pleocytosis this view has been almost generally abandoned. For syphilis there are now in existence the records of extensive researches in thirty cases (four adults with acquired syphilis and twenty-six congenitally syphilitic infants) conducted by Königstein and Spiegel (*Wien. klin. Woch.*, 1921, No. 24). The writers found meningeal changes in all these cases of positive findings in the fluid, but only exceptionally were changes found in the parenchyma itself.

¹ According to Lange endogenous protein originates from the disintegration of the spinal fluid cells, which in normal fluid arise from the endothelium, and in pathological (syphilitic) fluid from proliferated meningeal connective-tissue cells. I share the view with other writers (e.g., Goebel) that the disintegration of nerve tissue also plays a part in its origin.

to severe acute, or subacute, meningeal inflammations, such as pyogenic, tubercular, or syphilitic meningitis, or to the altered conditions of pressure in a part of the dural sac in which the movement of spinal fluid is obstructed. Endogenous protein consists chiefly of globulin, exogenous mainly of albumin; and accordingly with increased permeability there is a displacement of the normal ratio of globulin to albumin in the direction of an increase of albumin (cf. Chapter XIII).

Lange, supported by earlier researches of Mestrezat, thinks the first stage in a meningeal inflammation is due to a diminution of chlorides and an increase of sugar, with normal protein content of the spinal fluid. This is perhaps a first sign of hyperæmia and serous infiltration, and a very slight disturbance of permeability in consequence, which at first has only to do with the dialysable part of the blood plasma. With somewhat greater disturbance of permeability an increase of exogenous protein takes place prior to the increase of cells (Lange, Weigeldt). (The endogenous protein-increase of syphilis, however, as has been previously mentioned, makes its appearance later than the cell-increase, as numerous researches show.) The significance of the gold and hæmolysin tests will be found treated of in the chapters dealing with these reactions.

Now, what services can lumbar puncture render in view of the preceding remarks? Beginning with those lesions in which the meningeal affection dominates the clinical picture, i.e., the clinical forms of meningitis, experience shows that the typical clinical conditions can be associated with extremely different spinal fluid findings, e.g., turbid, purulent spinal fluid on one hand, perfectly normal fluid findings on the other.

The spinal fluid picture can, moreover, be influenced in many ways by differences in extent and position of the anatomical lesion, together with imperfect mixture of the fluid, which constitute a further limitation to the

interpretation of the spinal fluid findings in meningeal lesions (cf. Chapter I).¹

Much of the vagueness and difference of opinion on the subject of meningitis, especially in the otological literature, are probably owing to these circumstances. Thus references are occasionally made to pyogenic meningitis on the strength of the clinical findings, but notwithstanding clear spinal fluid; and the question is discussed whether for the diagnosis of pyogenic meningitis it is necessary to prove the presence of bacteria in the lumbar fluid as well as that of granulocytic pus. It is frequently pointed out that in meningeal infections the specific organisms are not always demonstrable in the spinal fluid, and that on the other hand, from the clear spinal fluid, bacteria (pneumococci) can sometimes be grown in pure culture, while circumscribed pyogenic foci are occasionally found in the presence of normal fluid. It would sometimes seem as if the terms pyogenic and infectious meningitis are employed as equivalent designations, and some writers think that the spinal fluid can be turbid without a suppurative meningeal infection. Thus Fleischmann believes that the lymph stream removes cell-elements from the neighbourhood of a cerebral abscess into the fluid. There appears to me no doubt that in cases of turbid fluid, granulocyte infiltrations of the meninges are regularly to be found (leaving out cases of abscess discharge into a ventricle), although they may occasionally evade the naked-eye examination.

Schottmüller has the merit of having been the first to force attention to the inconsistencies in the nomenclature of meningitis, and to have particularly stressed the view that the customary division of meningitis into purulent

¹ It is to be hoped that the (not really large) number of discrepancies between the spinal fluid findings and meningeal lesions may be still further diminished by more extensive fractional investigations of fluid, as previously mentioned, i.e., the withdrawal of large amounts in the sitting posture and investigation of at least the initial and the end portions, and perhaps by occasional puncture of the cisterna.

and serous meningitis is not justified by the facts, for a meningeal infection is not necessarily always accompanied by purulent spinal fluid, and a turbid fluid need not be the consequence of a meningeal infection, but can also be the expression of an irritation of the meningeal membranes which can be provoked by a neighbouring focus of inflammation. Schottmüller therefore classifies meningitis on ætiological principles into *meningitis infectiosa* and *meningitis sympathica* (the latter is also designated *meningitis comitans* and *meningitis symptomatica*). *Meningitis sympathica* is found, for instance, in otitis media, in inflammatory sinus-thrombosis, cerebral abscess, orbital diseases, suppuration near the orbit, &c. Conditions resembling the meningitic picture with inflammatory signs in the spinal fluid can also be provoked by mechanical and thermal influences (sunstroke), also by endogenous and exogenous toxins (worm-toxins, &c.).¹ Schottmüller subdivides *meningitis infectiosa*—and here the principle of classification is on the lines of pathological anatomy—into *meningitis infectiosa universalis* and *meningitis infectiosa circumscripta*. The latter is characterized by the presence in the region of the meninges of only single small inflammatory foci containing pathogenic organisms; the spinal fluid is perfectly free from disease organisms in consequence. (A very exact, and in certain circumstances repeated, investigation, however, is indispensable for a negative finding. The clinical picture must also be taken into account in the diagnosis, as a negative result in a case of suspected epidemic meningitis proves very little against the general infection of the meninges.) *Meningitis infectiosa circumscripta* is not infrequently found in septic diseases, in exanthemata (scarlet fever, measles), and in other infectious diseases, such as pneumonia, pertussis, mumps, &c.

¹ Thus toxic meningitis has been rendered probable in pulmonary tuberculosis, in which a parallelism between the exacerbations of the pulmonary disease and of the meningitis has occasionally been observed (Herschmann).

The principle on which Schottmüller's classification is based is a valuable one to apply in every clinical case. But it also has the defect that in cases of circumscribed infectious meningitis the factor of infection, which is the chief distinguishing character of the two groups, is not capable of being established by the investigation of the spinal fluid. In order to differentiate between the circumscribed infectious form and *meningitis comitans* recourse must therefore frequently be had—apart from the clinical picture—to the rest of the pathological conditions in the spinal fluid, above all to the degree of cell-increase. Another awkward aspect of Schottmüller's classification is that the circumscribed form of infectious meningitis is grouped together with general infectious meningitis; yet the former stands clinically much nearer to *meningitis comitans* in possessing a comparatively favourable prognosis, while the latter is usually much less favourable; and on the other hand, the relatively favourable clinical picture shown in acute syphilitic cerebro-spinal meningitis is perforce grouped with general infectious meningitis.

In the interest of a more suitable classification of the forms of meningitis I consider it commendable to classify according to the spinal fluid picture, and to utilize Schottmüller's viewpoint for the subdivisions. As already mentioned, a purulent and a serous form¹ of meningitis are distinguishable according to the findings in the spinal fluid. The characteristic signs of the two groups are: With purulent meningitis there is a milky turbidity of the spinal fluid; this arises from its high cell-content, which can amount to many thousands [per cubic millimetre]. The cells of the milky turbid fluid are always for the most part polynuclear leucocytes; an increase of mononuclear cells to such an extent that milkiness of the spinal fluid

¹ It should be stressed that this expression is not here used in the usual, though not always uniform, sense as the designation of a clinical picture.

would result, does not occur. On the other hand, a so-called sunmote-like turbidity ("sonnenstäubchenartige") occurs in the lymphocytic increases of greater degree, e.g., not infrequently in tubercular meningitis. In pyogenic meningitis there is, moreover, invariably a considerable increase of globulin and albumin, sometimes with fibrin clots, and usually an increase of pressure;¹ passage of amboceptor and complement, displacement of the gold test curve to the right, diminution of the chlorides, great diminution, up to absence, of sugar. *Meningitis serosa* is characterized by clear, faintly turbid spinal fluid, a greater or less degree of cell-increase in which as a rule the mononuclear elements predominate, moderate protein increase, usually a distinct, sometimes considerable, right displacement of the gold curve, moderate decrease of the chlorides, in slight forms of the disease also an increase of sugar.²

Of practical importance, especially on the prognostic

¹ But in certain circumstances the pressure can be very low in consequence of adhesions that effect an occlusion in the subarachnoid space, and after the withdrawal of small amounts of fluid the pressure can sink to zero; or it can occasionally happen that no outflow occurs whatever ("Punctio sicca") owing to great thickening of the purulent spinal fluid, or owing to exudate obstructing the orifice of the needle.

² As regards the term *meningitis serosa*, its employment is partly a question only of definition. If, with Fleischmann, we regard increase of pressure as its chief symptom, and refuse to apply the term in cases in which circumscribed inflammatory foci are present, which find their expression in the increase of cells and protein, the term must then be reserved, of course, for cases with isolated increase of pressure. I think it is more suitable to employ the term *meningitis serosa* as opposed to pyogenic meningitis for cases with slighter inflammatory signs in the spinal fluid, and to denominate the cases of undoubted increase of pressure, which with exact investigation certainly do not occur frequently, and also other anomalies, under the term spinal fluid congestion ("Liquorkongestion, Holzmann"). If the findings mentioned earlier, viz., abnormalities only of the dialysable ingredients, perhaps in combination with slight protein increase, which Lange regards as the first phase of the inflammation, should meet with confirmation on an extended scale, then the term *meningitis serosa* might come into consideration for this group of symptoms.

side, is the separation of *meningitis purulenta aseptica* from *meningitis purulenta infectiosa*. The difference between these two is that in the latter form specific organisms can be demonstrated in the fluid, while in the former they are absent, because in these cases the strong inflammatory reaction of the meninges is not due to a bacterial infection, but represents the mild form of meningitis from the ætiological standpoint.¹

Those conditions of the fluid, indicative of inflammation, which are differentiated by the presence of delicate fibrin clottings can be separated from serous meningitis under the term *meningitis serofibrinosa*. To this subdivision belongs above all tuberculous meningitis; but similar conditions occur occasionally in syphilitic meningitis, in circumscribed infectious meningitis, in sinus-thrombosis, and also, e.g., in uræmia. Such findings, however, are extremely rare, and in differential diagnosis, therefore, as a rule, practically negligible. But difficulties can sometimes arise. Thus some time ago I saw a case, which ended in recovery, of meningitis following an attack of influenza, in which all the spinal fluid findings, in every reaction—apart from the absence of bacilli—presented the typical picture of tubercular meningitis.

The term *meningitis purulenta infectiosa*, which adequately includes various forms of infectious meningitis (excepting the tuberculous form), e.g., epidemic, pneumococcal, staphylococcal, meningitis; and also the term *meningitis purulenta aseptica*—which may perhaps include the primary disease non-septic pyogenic meningitis in otogenous cerebral abscess, &c.—are, on the whole,

¹ Widal says that non-septic meningitis is characterized by well-preserved polynuclear cells, in contrast to infectious meningitis. Though not holding good universally, this assertion conforms so far to correct observation in that the increase of cells in the non-infected fluid, i.e., granulocytes, and the polynucleosis of the fluid to be mentioned later, usually sets in very acutely, and rapidly disappears, possibly by absorption, hence the fluid causes no serious damage to the cells.

terms that may be usefully employed, also for clinical purposes. The term *meningitis serosa* as a designation for a clinical picture is a source of difficulty, as slight inflammatory signs in the fluid can occur in general meningeal infections, as well as in circumscribed meningitic foci, and also as the expression of an "accompanying" meningeal affection.

Between the spinal fluid picture of pyogenic and that of serous meningitis there are of course gliding differences not only in the number of cells, but also in the variety of cell-elements. While nearly always in pyogenic meningitis only polynuclear cells are present, and in serous meningitis the lymphocytes usually preponderate to which in chronic lesions plasma cells, macrophages, &c., occur in addition, yet pleocytoses of moderate degree occur that do not lead to a milky turbidness of the spinal fluid, though nevertheless consisting for the most part of polynuclear leucocytes. Most of these are cases of rapidly passing, violent reactions of the meninges to injurious influences of a manifold nature, which have already been referred to in enumerating the causal conditions of *meningitis comitans*. Polynucleoses of the spinal fluid have been described in single examples of all these lesions, which are connected by gliding degrees with the severer pathological alterations of the fluid shown in non-septic pyogenic meningitis. Spinal fluid polynucleosis has thus been found in paresis (Pappenheim), in tabes and cerebral syphilis, in cerebral hæmorrhage, coal-gas poisoning (Hirtz), after sunstroke (Dopter and Dufour) ; strangulation (Pappenheim), in eclampsia and uræmia (Chauffard and others), and status epilepticus (Pappenheim).

In the presence of a clinical picture showing meningitic conditions, lumbar puncture is thus able to furnish the following information :—

(1) The spinal fluid shows the conditions concomitant with a *meningitis purulenta (infectiosa)*. Lumbar puncture

thus confirms the clinical diagnosis of acute pyogenic meningitis; the bacteriological examination of the fluid establishes its ætiology.

(2) The spinal fluid picture of a *meningitis purulenta aseptica* is present. Lumbar puncture supplies the important information that there is no general infection of the meninges, but that it is a case of *meningitis comitans*.

(3) The spinal fluid conditions associated with a *meningitis serosa* can be the expression of a circumscribed meningeal infection, or a reaction of slight degree to an injurious influence, which was mentioned as an exciting cause of *meningitis sympathica*. The diagnosis is one of *meningitis serosa* with addition of the causal agent, e.g., *meningitis serosa* in pneumonia, &c., with injury to skull (*meningitis serosa traumatica*), or cerebral abscess, &c.; or from a cause unknown (*meningitis serosa idiopathica*, sometimes referred to as acute hydrocephalus). The spinal fluid conditions in (1) and (2) must merge into the conditions of this group upon the abatement of the causal lesion. The conditions of group (3) can also originate primarily, then either returning to the norm, or in the case of aggravation of the lesion passing over to (1) and (2). The establishment of the pathological conditions in (2) and (3) assists, in some cases, the differentiation between a cerebral abscess and a pyogenic meningitis, &c.

(4) The conditions of a *meningitis serofibrinosa* are found. Clinically, this usually refers to a *meningitis tuberculosa*. The diagnosis is verified by established presence of tubercle bacilli in the fluid. But the presence in the fluid of the typical, delicate, veil-like, branched fibrin-clotting is, in general, sufficient for the diagnosis (see p. 129). A further character of tubercular meningitis is the excessive diminution of the chlorides (6 to 5 per thousand). It is known that, as a rule, round cells preponderate over the granulocytes, but the reverse is not infrequent. A suspicion of the presence of tuber-

cular meningitis is justified if, notwithstanding the absence of clots, there is a comparatively small protein increase (below three per thousand) in combination with a very great right displacement in the gold test (Lange).

(5) An isolated increase of pressure can occasionally be associated with meningitic symptoms. It can then be the first sign of commencing inflammatory changes in the fluid. The meningitic symptoms, however, can also disappear as a result of the relief of pressure. It is possible that the increase of pressure in the spinal fluid is frequently, or regularly, related to the disturbance of the dialysable part of the fluid previously mentioned. Investigations are lacking upon this point. This disturbance would then have to be included in this place, or between (4) and (5).

(6) Notwithstanding the presence of the clinical symptoms of meningitis, no pathological alterations are found in the fluid. Diagnosis—meningism (after-symptom following lumbar puncture, though sometimes a *meningitis serosa* is present—observed in some cases after salvarsan injection and in epilepsy). The cases in group (5) are also often designated as meningism.

Lumbar puncture thus enables a general meningeal infection to be excluded from the diagnosis in a number of cases, notwithstanding the presence of the clinical meningitic picture. Conversely, an investigation of the fluid indicates in a large number of cases the encroachment of an infectious disorder on the meninges, even when the clinical signs of meningitis are absent. This is of great importance in the case of lesions in which the danger of meningeal infections is especially imminent, as in diseases of the ear and injuries to the skull.¹

¹ Pathologico-anatomically speaking, a meningitis can be already present in such cases, just as is the case in the spinal fluid alterations of a syphilitic character which show no clinical signs, or occasionally in inflammatory states of the fluid unaccompanied by clinical symptoms of meningitis, as, e.g., after carbonic oxide poisoning. It is, however, advisable in such cases, if no clinical meningitic picture

The value of lumbar puncture during the observation of cranial injuries is rendered obvious by Payr's remark, who compares it with a barometer reading, as it affords information on the prevailing variations and supplies a warning sign in a commencing infection. Tilmann also attributes the greatest importance to lumbar puncture in injuries to the skull. He says: Every increase of albumin indicates the presence of inflammatory or regenerative changes going on within; stronger degrees indicate cerebral abscess. An increased pressure that falls little or not at all speaks for an intracerebral lesion, and with simultaneous high albuminous content, for cerebral abscess; with low albuminous content for an inflammatory cyst, and very small increase for a retention cyst. If the increased pressure falls rapidly, then a meningitis serosa is usually present.

A special significance to lumbar puncture is given by Clairmont in cases of recent cranial injury. He considers that in cases which show no increase of pressure, but only a very small amount of bloody spinal fluid, or an outflow of pure blood, that the indication is given for the performance of trepanation of the skull, which stimulates the disturbed production of cerebro-spinal fluid.

A special differential value to lumbar puncture obtains in otology with reference to the questions raised: on the one hand, pyogenic meningitis with or without bacteria? and on the other, normal fluid or inflammatory

is present, to avoid using the term meningitis, and in its place to state, for instance, the following diagnosis: sinus-thrombosis with non-septic purulent spinal fluid; sunstroke with polynucleosis in the spinal fluid; traumatism of skull with serous inflammatory alterations in the spinal fluid; chronic nephritis with increase of pressure in the spinal fluid, &c. The expression syphilitic meningitis will then be reserved for cases with the clinical signs of meningitis. The term neurorelapse, often employed for inflammatory signs of the fluid in syphilis, is unsuitable, because similar changes also occur in cases not under treatment. Nor do I consider Nast's expression, spinal fluid syphilis ("Liquorlues") particularly fortunate.

signs in the same? In general, the prognosis is comparatively favourable for an affection with turbid but sterile spinal fluid, which is found, e.g., in cases of chronic otitis with attenuated organisms, or not seldom in cerebral abscess; while the establishment of a pyogenic infectious meningitis, often found in acute lesions, usually means an unfavourable prognosis. The occurrence of spinal fluid abnormalities in diseases of the ear and the neighbouring parts is not only of great diagnostic importance as a sign of endocranial complication, or of a threatened, or already established, encroachment on the meninges—thus in suspected cerebral abscess an even slightly positive finding gives support to the diagnosis—but the abnormal fluid can also provide leading indications for further therapeutical procedure. Certainly the findings can at times be deceptive; thus the occurrence together of a lesion of the middle ear with a tumour of the brain, the latter giving rise to a slightly positive finding, can be the occasion of an erroneous diagnosis of cerebral abscess (Fleischmann).

With regard to particular endocranial complications, it follows from what has been said that varieties of meningitis can occur in every such case. Here there are no really characteristic findings, not even in cerebral abscess in which some writers always find abnormalities (Knick), others sometimes negative fluid (Voss), some find usually clear fluid with much increase of cells of a more lymphocytic than polynuclear character (Fleischmann), while Fremel, in agreement with Alexander and Neumann, considers a turbid sterile fluid (fourteen times in seventeen cases) as characteristic for cerebral abscess. From the literature it would appear that turbid fluid is much more frequent in otitic cerebral abscesses than in abscesses after cranial injuries. This is probably due to the circumstance that otitic cerebral abscesses approach the cortex, or, as Neumann has pointed out, that the

ventricle wall shows malacia. The interesting, though practically unimportant, observation of Borries may be mentioned, that the diminution or disappearance of spinal fluid abnormalities, notwithstanding the continuance or aggravation of the clinical symptoms ("benign cerebro-spinal fluid picture") speaks for cerebral abscess. (Fremel expounds that in practice before such a picture one cannot usually spend so much time beholding it.) Uncomplicated sinus-thrombosis is usually unaccompanied by abnormalities in the fluid (Knick). In this respect it is very significant that the fluid can be normal in spite of the clinical symptoms of meningitis, from which the presence of meningitis can be inferred (Fremel). The appearance of inflammatory signs in the fluid in cases of sinus-thrombosis heralds the encroachment of the infection on the meninges, and is an unfavourable foreboding. Extradural abscesses show analogous behaviour (Knick). The spinal fluid findings are of great importance in labyrinthitis. In uncomplicated labyrinthitis the spinal fluid findings are normal (Knick, Fremel) and permit, if still kept under observation, a delay of operation (Knick); the appearance of pronounced if but slight abnormalities in the fluid, even in the absence of clinical symptoms—in one of French's cases there were spinal fluid alterations to a high degree which were not manifest in the clinical picture—indicates the attack of the infection on the meninges and renders operation necessary (Knick) as the prognosis becomes unfavourable if an extensive infection of the meninges has taken place. (Fremel's observations confirm this.)

Leaving the confines of meningitis (in the clinical sense), lumbar puncture is found to be of further diagnostic value for differentiating, firstly, between organic and functional nervous diseases; and secondly, between non-infectious and infectious organic diseases of the central nervous system.

The available experience in this respect is as follows:—

Functional nervous disorders are not accompanied by abnormalities of the spinal fluid.

Organic non-infectious diseases of the nervous system show as a rule no pathological spinal fluid findings; in a comparatively small number of cases alterations in the fluid occur, usually only of small degree. Thus slight changes are found in about 15 to 20 per cent. of cases of true epilepsy and in cerebral hæmorrhages and softening. An abnormal spinal fluid occurs in the majority of cases of cerebral tumour, rarely a slight increase of cells and albumin; usually only a slight albumin increase which may be combined with a small right displacement—1:80 and 1:60 (Lange). An important symptom in the differentiation between tumour of the brain and serous meningitis is the rapid fall of pressure after the withdrawal of small amounts of fluid. In tumour a gradual fall of pressure speaks for the pressure arising less from the tumour than from the accompanying hydrocephalus. (Somewhat more spinal fluid can then be withdrawn without danger.) Lange stresses the importance of xanthochromia for the diagnosis of tumour of the brain (cf. Chap. VII); the xanthochromia is more constant as opposed to the findings when only a single hæmorrhage has taken place. A finding which is perfectly analogous to that of tumour can also be afforded by hæmorrhagic internal pachymeningitis, when for differential diagnosis recourse must be had to the clinical signs.

Infectious diseases of the cerebro-spinal parenchyma are frequently accompanied, particularly in the fresh stages, by considerable inflammatory signs in the fluid.

(1) Lumbar puncture generally affords but little assistance in the differential diagnosis between functional and organic nervous disorders, as in the majority of cases the organic basis of nervous disturbances can be confirmed by the clinical examination. But it has some value for

the formation of an opinion in nervous disturbances after cranial injuries. According to Tilmann it affords a "very safe guide" for confirming or dispelling a suspicion that complaints are counterfeited.

Leaving aside cranial injuries, a positive spinal fluid finding with ambiguous anamnesis and insignificant clinical symptoms can strengthen the diagnosis of an organic ailment, e.g., *encephalitis lethargica economo*, poliomyelitis, an abscess of the brain, as opposed to functional disturbances. In rare cases a pathological finding in the spinal fluid may support the diagnosis of epileptical as against hysterical attacks. Occasionally the establishment of pathological changes in the fluid can corroborate the suspicion of carbonic oxide poisoning if the gas has been eliminated by ventilation of the lungs—frequently xanthochromia and pleocytosis (Legry and Lermoyez), and sometimes polynucleosis, may occur in such cases. Lumbar puncture can also sometimes prove useful in differentiating between purely toxic cerebral disturbances in general infections, as e.g., in influenza and encephalitis (Grosz and Pappenheim).

(2) The differential value of lumbar puncture as between infectious and non-infectious nervous diseases is, on the clinical side, chiefly concentrated on the differentiation between nervous syphilis and other organic ailments. Here a positive Wassermann reaction in the fluid is able readily to dispel any doubts. (The rare exceptions of non-syphilitic organic disease in a syphilitic person, and combination of a syphilitic with a non-syphilitic affection of the central nervous system, scarcely come under practical consideration.) But also with a negative Wassermann reaction in the spinal fluid the degree of inflammatory alteration in the fluid usually enables a differentiation to be made, as syphilis—excepting purely vascular disorders and older circumscribed forms of meningo-encephalitis or meningomyelitis, and the extremely rare primary degenerations of the cord—in

contrast to the non-syphilitic diseases of the central nervous system, is usually accompanied by extensive pathological alterations of the spinal fluid.¹

The differentiation between syphilis and other infectious organic diseases is not usually a source of great difficulty, for the non-syphilitic infections of the central organ usually take an acute course, and therefore from a clinical viewpoint do not come into consideration for differential diagnosis, and also because the chronic forms as a rule only show considerable alterations of the fluid in the early stages. Thus in multiple sclerosis, the infectious character of which, indeed, is not yet quite established, abnormal spinal fluid reactions are found in most cases, though the alterations are usually only slight in degree—I found with especial frequency only a slight increase of protein—and only in a few cases do they reach the intensity of the average alterations of the fluid in syphilis. Should there be any difficulty, therefore, in differentiating between multiple sclerosis and a syphilitic lesion, only strong indications in the fluid of inflammatory conditions can be utilized with certainty (more than fifty cells to the cubic millimetre), and medium indications (twenty to fifty cells) with a certain degree of probability, to support the diagnosis of a syphilitic lesion.

The clinical pictures of epidemic encephalitis but rarely give rise to differential considerations as against a

¹ Such changes can be present and can prove the presence of syphilis, even if the Wassermann reaction in the serum is negative. Spinal puncture is especially important precisely in cases of suspected syphilis in which the serum reaction is negative, and the syphilitic ætiology of a nervous disease should not be rejected on account of a negative serum reaction. It is, for example, gross negligence when, as in a case seen by me, a patient with ocular muscle paralysis, examined by a professor of neurology and a professor of ophthalmology, and, owing to negative Wassermann reaction, is treated for months merely with galvanization and small doses of iodine, until a lumbar puncture, caused to be performed by another quarter, established the syphilitic basis of the disturbance. Such patients were better off in the pre-Wassermann era, as in those days they would have received anti-syphilitic treatment in any case.

syphilitic lesion. In its early stages it shows spinal fluid abnormalities with great regularity, which usually disappear in the further progress of the disease. There is as a rule increase of pressure, usually only a moderate increase of cells, which can, however, be quite considerable (even turbid spinal fluid is found now and then), and generally a slight increase of protein in comparison to the cell-increase (the French speak of a "dissociation cyto-albuminique," which they consider to be present if with a maximum content of 0.4 per thousand there are 30 to 100 cells to the cubic millimetre). The gold reaction shows the syphilitic type in the most varying degree, sometimes to the extent of the paralytic curve. A regular symptom is the increase of sugar. In a few cases in the acute stage a positive Wassermann reaction has been found in the spinal fluid.

Typhus fever, which is generally associated with very great pathological alterations in the fluid, is also to be placed in this group. Besides cell and albumin increase, the spinal fluid in this disease almost invariably gives a positive hæmolysin reaction, and frequently a positive Weil-Felix reaction (agglutination with *Proteus* X19).

Very inflammatory conditions in the spinal fluid can thus, according to foregoing remarks, be brought into requisition in doubtful cases with the greatest degree of probability for the diagnosis of nervous syphilis; while negative or insignificant findings are against a syphilitic ætiology and in favour of the diagnosis of another ailment, e.g., multiple sclerosis, spastic spinal paralysis, encephalomalacia or hæmorrhage with a non-syphilitic basis. In forming an opinion on absent or slight alterations of the spinal fluid, however, reserve should be exercised, and one must not be surprised if in some cases lumbar puncture should not give satisfaction as, e.g., in the differential diagnosis between an old case of cerebral syphilis and a cerebral arterio-sclerosis.

Combined with careful consideration of the clinical

picture, lumbar puncture can also be made use of in differentiating between the later forms of nervous syphilis. The significance of the Wassermann reaction in the spinal fluid,¹ the gold test and the hæmolysin reaction have been reviewed in this connection in the special chapters. As regards the inflammatory symptoms, the fluid in fresh cases of true nerve syphilis (gummatous and meningo-encephalitic or meningo-myelitic forms) usually shows greater pathological changes than in tabes² and general paresis³; the pathological reactions in paresis or tabes-paralysis are as a rule more strongly marked than in tabes, and they show less tendency to diminish than in the latter; finally, in old cases of true nerve syphilis and older cases of tabes the smallest changes in the fluid are found, and not infrequently the findings are entirely negative.

Weak reactions of the fluid in a case of tabes with psychosis may thus be interpreted as being against an additional paresis, while an increase in degree of the reactions will afford support to such a supposition. In

¹ It may be mentioned in passing that the Wassermann reaction has occasionally a certain value in that a negative finding is against the diagnosis of paresis. In paresis the serum reaction is almost invariably positive, in other forms of late nervous syphilis in 50 to 70 per cent. of the cases.

² Lange states the following spinal fluid findings to be characteristic of tabes incipiens: Albumin *ca.* 0.25 to 0.40 per cent., syphilitic indention in the gold test, increase of round cells without granulocytes, negative Wassermann reaction in spinal fluid—a finding which coincides perfectly with that in many cases of multiple sclerosis. The differential diagnosis is, according to Lange, outside the spinal fluid finding, viz., in the Wassermann reaction in the serum; this is negative, of course, in multiple sclerosis, while in incipient tabes it is said to be negative in inactivated serum, but ++++ in active serum.

³ An interesting observation of Plaut and Steiner may here be incidentally referred to. After inoculations which they had undertaken for therapeutic purposes they found in the spinal fluid of paresis after the entry of *Spironema obermeieri* ("Recurrentzspiro-somen") a considerable increase of the cell content—beyond one thousand cells—and an increase of protein; while in a case of dementia præcox only a large cell-increase took place.

differentiating between cerebral syphilis and paresis, an increase of cells amounting to many hundreds, particularly in combination with a considerable protein increase and much variation between both reactions, may be taken to favour the former diagnosis; moderate increase of cells together with moderate or comparatively large protein increase speaks in a fresh case more for a paresis; inconsiderable cell and protein increase with longer duration of the disease more for cerebral syphilis. In many cases the influence of antisyphilitic treatment on the spinal fluid, generally greatest in true nerve syphilis, less in tabes, and least in paresis,¹ can be of significance. Exceptions to these rules are found in every direction; even in paresis, which is most constantly associated with alterations in the fluid, isolated cases have been observed in which spinal fluid abnormalities were absent, notwithstanding the progressive advance of the disease. (This has often been recorded of stationary and atypical cases.)

The spinal fluid findings can also now and then be of significance in the sometimes specially difficult differentiation between paresis and the more rarely occurring pathological disturbances due to a syphilitic affection of the small vessels of the cortex. Although parietic spinal fluid characters are shown in some cases of this disease, entirely negative or slightly positive findings can also occur, or when the meninges are greatly involved considerable variations in the reactions can occur.

In the purely arteritic forms of cerebral syphilis the fluid shows only slight alterations. The Wassermann reaction is usually positive in large doses, not infrequently negative, and the cell-increase is generally moderate, or it can be absent; the same applies to total protein increase. In older cases especially, the spinal fluid finding is not infrequently negative.

¹ Although Wagner-Jauregg have achieved extensive favourable modifications of the spinal fluid in the malaria treatment of paresis which they have inaugurated.

That severe meningitic spinal fluid abnormalities usually occur in the early stages of nerve syphilis, and in neuro-relapse and acute syphilitic cerebro-spinal meningitis, has already been mentioned. There is generally a great increase of cells, often running into thousands, with granulocyte increase, though with a strong preponderance of mononuclear elements; usually a not very considerable protein increase and a right displacement of the gold curve, but sometimes with a curve of the syphilitic-parietic type. The positive Wassermann reaction—though often only appearing with large doses—distinguishes the spinal fluid finding from that of non-syphilitic meningitis, though not invariably. The findings usually revert quickly, especially with suitable treatment, to the slighter abnormalities of latent states, or also to the norm.

In regard to congenital syphilis, it really shows no essential deviations from the acquired variety. Paresis gives typical findings; cerebro-spinal syphilis is mostly accompanied by changes in the fluid, as with adults; on the other hand, the findings in epilepsy, hydrocephalus, idiocy, deafness of central origin and syphilitic aetiology are usually insignificant or negative. It is noteworthy that pathological reactions occur in a considerable percentage of cases of Mongolian idiocy (Nonne). Positive findings are often found in congenitally syphilitic infants, though they usually diminish rapidly. Striking is the frequent occurrence of an isolated Wassermann reaction in the fluid, which is extremely rare in adults. On this point, however, the general opinion is not unanimous; thus Frank on the contrary finds frequent negative Wassermann reaction with otherwise positive findings.

Special consideration in the interpretation of pathological spinal fluid findings is necessitated by the fact that in syphilitic pupil disturbances inflammatory signs in the fluid with positive Wassermann reaction are of very frequent occurrence, and are often as pronounced as in

tabes and paresis. With paresis or tabes suspects, therefore, the possibility should invariably be kept in view of a combination of pupillary disturbance with neurasthenia, pupil disturbance with mania, pupil disturbance and neuritis, and so on.

The "compression syndrome" in the spinal fluid, to which Nonne first called attention in 1908, is of considerable diagnostic value. It is characterized by an increase of albumin, together with a normal, in some cases slightly increased cell-content (the "dissociation par hyper-albuminose" of the French writers). The protein increase often attains enormously high degrees (up to several parts per cent.), but is then always combined with xanthochromia (cf. end of Chapter XIV), and spontaneous coagulation sometimes takes place. Queckenstedt's symptom was a valuable addition to the syndrome (see end of Chapter IV). The symptom is a reliable sign that the subarachnoid space is occluded at some spot above the site of the puncture; the isolated protein increase similarly confirms the diagnosis of an occlusive lesion when the clinical symptoms point in that direction.

The syndrome can supply no information on the nature of the lesion. It is found to occur in extradural and intradural and even intramedullary tumours, in acute transverse myelitis—in the last two cases probably due to collateral oedema—tubercles, Pott's disease, traumatic fractures of the vertebræ, cysts, echinococcus, cysticercosis, funicular myelitis, in diffuse and disseminated necrotic conditions; in the case of a meningitic lesion characterized by cell-increase, a positive Queckenstedt's symptom or a very high degree of protein increase permits the inference of obstructing adhesions of greater or less degree.¹ Neither does the syndrome give any

¹ As according to this the so-called compression syndrome need not only be conditioned mechanically but also as a result of inflammation—I called attention to its inflammatory origin long before Lange—the use of the more general term *spinal fluid stasis* ("Liquorstase")

information as to the position of the lesion unless a puncture can be performed below and above the obstruction; above it is found normal spinal fluid; this fact enables a decision to be arrived at in an operation in which the lesion is not always located at once as to whether an opening has been made too high or too low. Queckenstedt's symptom can also serve to point the way during the operation; if one should be above the obstruction compression of the veins of the neck induces a convexity of the dural sac, while if below it no change is seen.

According to Queckenstedt, the isolated protein increase observed by him in some cases of sciatica and polyneuritis is ascribable to a simple non-inflammatory disturbance of circulation due to changes in the tissues around the nerve roots and leading to a passage of blood-albumin into the spinal fluid (Queckenstedt stresses, incorrectly according to my experience, that only the total protein is increased, not the globulins too). The proof of this alteration in the fluid could in certain circumstances be of value for establishing the organic basis in a case of sciatica. According to Heinze, who confirms the occurrence of protein increase in sciatica, its presence permits the differentiation between root-sciatica and one arising from the cord.

In forming an opinion in cases of recent spinal injuries, the severity of the spinal fluid abnormalities has been recognized as symptomatic of damage to the cord—e.g.,

suggested by Lange is to be recommended. This can owe its origin to: (1) Purely mechanical conditions either by compression or from adhesions following in the wake of inflammation; (2) purely inflammatory conditions, e.g., in meningitis, which can lead to an extraordinarily large swelling sufficient to engrasp the cord ("Umklammerungsmeningitis," Lange), or to adhesions, which become loosened again, thus giving rise to a changing picture; or (3) to mixed forms with secondary inflammation, in which although a cell-increase occurs, it is small in comparison with the protein increase, and is also small compared to the cell-increase of the purely inflammatory form.

inflammatory signs with admixture of blood (Schulz and Haucken), absence of the pulsatory variations of spinal fluid pressure, dura-laceration (Knauer).

The spinal fluid abnormalities occurring in other diseases are not of any great diagnostic value. Only out of interest it may be remarked that herpes zoster, the disease of the spinal ganglia designated by Head as *poliomyelitis posterior acuta*, is regularly associated with considerable inflammatory alterations of the fluid.

Pathological alterations of the fluid in the various general infections, among which typhoid fever often shows considerable abnormalities, usually amount from the practical viewpoint to only accessory findings. Also in non-nervous syphilis the chief value of lumbar puncture is not on the diagnostic side. Cases now and then occur with negative anamnesis and negative or doubtful serum reaction in which the diagnosis of latent syphilis may be strengthened, the syphilitic basis of headaches established, and a positive spinal fluid finding is said occasionally to render possible the difficult differentiation between a residual sclerosis or a monorelapse and a reinfection (Königstein).

The prognostic value of lumbar puncture in the various forms of meningitis, cranial injuries, diseases of the ear, follows directly from the preceding remarks.

In recent years, since continually accumulating investigations have established the occurrence of spinal fluid changes in the early stages of syphilis, also in the absence of nervous disease, the attention of numerous workers has been directed to their prognostic value. The results in this direction may be briefly summarized.

The first symptom in the spinal fluid which occurs shortly after the syphilitic infection has been observed by some writers (Dreyfus, Rost, Fleischmann) to consist in an increase of pressure, which can be regarded as a sign of spinal fluid increase and the harbinger of a more inflammatory reaction of the meninges. A slight increase

of cells also occurs, in some cases already in the sero-negative stage, but not earlier than the fourth week of the disease; and a positive gold reaction has also been observed among the early symptoms by some writers. A slight protein increase sets in after the appearance of glandular swelling in some cases of sero-negative syphilis. A positive Wassermann reaction is apparently not found in the spinal fluid at this stage. (The few positive results obtained by Fleischmann stand in contradiction to the other data found in the literature of the subject.) The frequency of the spinal fluid abnormalities (always slight) in the sero-negative stage of syphilis probably does not exceed 8 to 10 per cent. As soon as the serum reaction has become positive the spinal fluid alterations rapidly increase in number and intensity, and, according to the researches of Königstein and Goldberger, they attain their acme towards the tenth month, when they gradually decline.

In the majority of all cases of syphilitic infection the spinal fluid is undoubtedly altered within the first year. (And it has been repeatedly proved that during this period lesions of the meninges become established.) But these changes are largely of a transient character; they disappear, either with or without treatment, in such a manner that in the later latent stage spinal fluid abnormalities are only found—excluding, of course, the cases with clinical neurological symptoms—in about 20 per cent. of the cases, of which about one half are severe. Occasionally, however, anomalies of the spinal fluid do not appear for the first time until the second year has elapsed (Königstein, Mucha and Satke). Subjective complaints in the early stage of syphilis are much more frequently found in cases with a positive than in those with a healthy spinal fluid finding, but they also occur in the latter.

Different views obtain on the simultaneous occurrence of spinal fluid abnormalities with definite clinical sym-

ptoms. Some writers found spinal fluid alterations specially frequent in exanthemata; Königstein and Goldberger, and Leyberg found them to occur more often in the papular than in the macular eruptions, while others (Hauptmann, and more recently Mucha and Satke) point out that latent, and especially latent sero-positive cases, afford a higher percentage of spinal fluid abnormalities than cases with general or local syphilitic symptoms. It appears to be established that an unusually high percentage of spinal fluid changes is found in alopecia. With reference to the tertiary stage the data are strongly discordant. While some writers have never, or hardly ever, seen spinal fluid alterations, which they associate with the rare occurrence of nervous disturbances in syphilis III, others found abnormal conditions with great frequency. Kyrle ascribes these divergences to the capacity for spontaneous recovery in the case of spinal fluid abnormalities in gummatous syphilis.

With regard to the influence of antisyphilitic treatment on the spinal fluid—intraspinal treatment is not included in these remarks—it is no doubt a well-established fact, which Gennerich has above all specially emphasized, that in the early secondary stage every kind of treatment in general has a provocative effect, if but transitory. A comparison, however, of treated and untreated cases in which the infection dated back a year or more, shows on the other hand that in the treated cases the spinal fluid abnormalities are less frequent (Fleischmann, Mucha and Satke). A very energetic treatment in the quite early stage is evidently specially effective, though a success may still be achieved at a later stage, in the course of the first two years after the infection (Finger and Kyrle), while after this period it is difficult to effect an improvement. Slight pathological alterations in the fluid are said in general to be more subject to therapeutical influence; cases in which spontaneously or under treatment the alterations are specially violent are more inclined to recovery

("Rückbildung") than cases in which the anomalies show a lingering development (Finger and Kyrle). According to Kyrle, relapses are comparatively frequent if the gold reaction remains positive while the fluid is negative to other reactions.

Now referring to the prognostic significance of spinal fluid alterations in respect of later nerve syphilis, the results are at the present time still in a very unreliable state, owing to there being very little utilizable case-material at our disposal, i.e., cases controlled continually over a period of years. In this direction two works of Meyerbach and Fuchs are of great importance. Above all, they strengthen the doubts expressed by many workers in recent years as to the validity of the often dogmatically expressed view that metasyphilis invariably develops in a lingering manner ("schleichend") from early meningitis. F. Lesser even thinks that syphilitics with negative spinal fluid findings in the early stage are far more liable to become stricken later with tabes or paresis, and he expresses the decided view that the foundation for metasyphilis ("Keim zur Metalues") is only laid in the late period. The works referred to show that on the one hand a not inconsiderable number of syphilitics who show normal spinal fluid in the later latent period do get metasyphilis later on, and that on the other hand a surprisingly large number of syphilitics who present even severe spinal fluid abnormalities in the period of later latency remain spared, over many years' observation, from metasyphilis. (Particularly noteworthy is the low frequency in the female sex.) For the rest, I have drawn the conclusion from the data furnished by Fuchs, partly opposite to the writer's own conclusion, that syphilitics who present severe spinal fluid abnormalities in the period of later latency are comparatively in greater danger; while an isolated protein increase is in general not of unfavourable prognostic significance. It goes without saying that we have no possibility of individually

sifting out the endangered persons. But it must be stressed that our knowledge in this direction is still insufficiently founded, and that a solution of these problems must await the accumulation of extensive data based on systematic spinal fluid investigations.

If therefore in the present state of knowledge we have no right to overestimate the importance of spinal fluid alterations in individual prognosis, and as a guide for therapeutical measures, yet a certain duty is imposed on us, in the absence of reasons to the contrary, to undertake all possible steps towards improving the condition of the fluid when a pathological finding is present. The most important moment for investigating the state of the spinal fluid is probably that of the intended completion of the chronic-intermittent treatment—according to Kyrle and Mucha at the end of the second year, because then on one hand the period of most frequent spinal fluid abnormalities has been passed, and on the other, because slight and transient changes of the fluid have already diminished. With a negative finding it may be recommended to investigate the fluid again in a year, or perhaps two years, after the completion of treatment, preferably after salvarsan provocation (Gennerich); should alterations be present, and the spinal fluid is to be restored to condition, it will not be possible to evade further investigations. During the period of treatment an examination of fluid will only be indicated on the appearance of symptoms of early nerve syphilis. Gennerich considers a control of the spinal fluid of importance after the first course of treatment, so as to obtain a picture of any provocation that may be present. A spinal fluid examination is greatly to be recommended in older cases that it is desired to bring under treatment, and which have not received sufficient previous treatment. And if in an older case suspicious nervous symptoms make their appearance an investigation is dictated unconditionally. It may be mentioned that Nichols has suggested the

requirement of a spinal fluid examination as a condition for marriage.

On the prognostic significance of spinal fluid alterations in isolated syphilitic nervous symptoms, e.g., stiff pupil, it is possible to regard a negative finding as a favourable sign, while in the extreme frequency of positive findings—in 80 per cent. of cases of isolated loss of pupil-reflex—as Nonne rightly observes, one must beware of prophesying the course of a severe disturbance of the central nervous system in individual cases, in spite of the presence of even considerable alterations in the fluid. But according to the researches of Dreyfus the bearers of such pathological changes are endangered in a high degree, so that such cases necessitate continuous intermittent treatment.

In the region of clinically manifest attacks of late nerve syphilis, as in spinal syphilis, if the spinal fluid abnormalities also diminish with the clinical symptoms, it is generally possible to give a favourable prognosis, while with the persistence of pathological findings the danger of relapses must be reckoned with. Similar remarks hold good for the other examples of true nerve syphilis, and also of tabes with the exception of very advanced cases, which when continually progressive not infrequently show negative or only slightly positive findings. Increase or decrease in the findings is of far less significance as long as they remain pathological. The clinical course of the disease is very largely not parallel with these variations, and many writers fall into the error of schematizing treatment too closely according to the spinal fluid picture.

CHAPTER XXI.

THE THERAPEUTICAL APPLICATIONS OF
LUMBAR PUNCTURE.

LUMBAR puncture is employed for therapeutic purposes (1) for the relief of pressure, and (2) with the object of introducing medicinal agents into the subarachnoid cavity ; sometimes also in combination with the relief of pressure. In a few cases lumbar puncture has been performed in order to employ the fluid therapeutically by means of subcutaneous injections, e.g., in epilepsy, in epidemic meningitis.

Relief of pressure by the performance of lumbar puncture can be a means of saving life in some cases of recent cranial injury,¹ in apoplectic attacks, in severe states of uræmia, in pachymeningitis, in acute infectious meningitis.

Quincke recommends its employment in all cases of increased brain-pressure dangerous to life in which an extravasation is in question as the cause or part cause. As it is just in some cases in this group that lumbar puncture is not without danger (cf. Chapter III), a particularly conscientious consideration of the indications and special precaution in performing the puncture are essential.

It is thought that the therapeutical effect of reducing the pressure is to unload the paths of outflow of the compressed blood- and lymph-vessels, which is able in certain circumstances to restore normal conditions of circulation and thus to break down a *circulus vitiosus* ; in

¹ Clairmont found that in cases in which after the injury the production of spinal fluid is diminished or arrested (see p. 42), the favourable therapeutical effect only sets in after trepanation.

certain cases injurious substances are removed with the spinal fluid, and possibly reactions of a biological nature tending towards recovery are thereby induced.

A beneficial result following lumbar puncture has been observed in a great number of diseases. Thus its favourable influence on the course of typhus fever (Müller and others), in the cerebral disturbances of typhoid fever (Schottmüller and others), insolation and œdema of the brain has been stressed. The successes achieved by lumbar puncture in chorea minor, delirium tremens, conditions of excitement during psychoses, pruriginous affections, bronchial asthma, injuries to the optic nerve following influenza, choked disc due to various causes, disturbances of vision from methyl alcohol poisoning, have all been highly spoken of. In convulsions due to whooping-cough in children, in status epilepticus, in eclamptic and uræmic convulsions, in eclamptic, uræmic and diabetic coma, lumbar puncture often has a beneficial, sometimes life-saving, effect. It is also said to be attended with success in hydrops gravidarum in the pre-eclamptic stage; in such cases the puncture is sometimes followed by a copious urinary flow, which is set down to vasomotor influences at the periphery, which are said to be an important factor in its occurrence and for the reduction of the œdema (Mayer).

Different forms of headache, as in migraine, in hydrocephalus angioneuroticus (Quincke), in tuberculosis (Fritsch), in polycythæmia, chronic nephritis, &c., are favourably influenced. In diseases of the ear successes have been obtained in the conditions known as Bárány's syndrome, the pathologico-anatomical basis of which is not generally recognized, viz., circumscribed collection of cerebro-spinal fluid in the cerebello-pontile angle, pressure-increase due to adhesions at the margins of the cisterna, and transmission of the pressure-increase into the labyrinth along the sheaths of the auditory nerve. A favourable influence of lumbar puncture has been

repeatedly observed in vertigo and auditory disturbances, e.g., in traumatic affections of the labyrinth; but the effect is sometimes unfavourable (Ruttin). I saw a patient with vertigo and bilaterally deranged hearing who periodically requested a lumbar puncture. Permanent successes are said to have resulted from repeated lumbar punctures at intervals of three to ten days in a few cases of chronic hydrocephalus. In cerebral tumour, if palliative operation is refused, the favourable influence of lumbar puncture on the secondary hydrocephalus can bring temporary relief. Similarly in occlusive lesions of the vertebral canal lumbar puncture can effect an improvement in some of the symptoms (Nonne). The alleviating effect in the pain of sciatica is constantly being brought to notice.

In syphilis a good effect is often observed after lumbar puncture in the headache of the early stages, in the general symptoms of neurorelapse, and in early syphilitic meningitis, in syphilitic papillo-œdema, and in symptoms of cerebral œdema after salvarsan.

In traumatic injuries to the brain, especially in protrusion, some writers recommend the systematic employment of lumbar puncture. Also in cases of chronic complaints after cranial injuries, repeated puncture at intervals of several weeks sometimes has a good effect. Demmer has suggested the performance of a lumbar puncture in the sitting posture at each change of bandages in cranial injuries. He claims that owing to the better opening up ("Entfaltung") of the wound the course of the injury can be better kept under observation, and that the advance becomes slackened, and there is a readier expulsion of splinters, &c.

More recently the drainage of spinal fluid recommended as a supplementary support in the intravenous salvarsan treatment appears to be more extensively adopted, viz., the withdrawal of 20 to 25 c.c. of fluid ten minutes before the injection, thus promoting the passage of the

medicament into the spinal fluid. Fleischmann recommends the employment of this drainage in his method of "inundating" the blood with anti-streptococcus serum in infectious meningitis.

Therapeutical injections of specific or non-specific antibodies, and of medicaments, into the lumbar cavity have been employed in different diseases; medicaments are used partly for irrigation purposes only, sometimes they are left in the subarachnoid space.

Injections of serum, particularly of anti-meningococcus serum, are extensively employed in the treatment of epidemic meningitis; they should be performed with a syringe attached to the cannula by means of a rubber tube. Injections of 10 to 20 c.c. in increasing amounts, if desirable up to 40 c.c., are made daily at first, later less often. Before the injection it is necessary to remove a larger amount of fluid, at least 30 c.c. more than it is intended to inject, for the purpose of relieving the pressure. Serum intended for subcutaneous injection and containing carbolic acid is to be avoided. Tetanus antitoxin is administered intraspinally in daily doses of 100 to 200 antitoxic units. In severe cases of diphtheria Bingel, followed by Weintraud, have recommended the injection of antitoxic serum up to 6,000 units. Gmelin saw epileptics freed for a longer period from attacks after the injection of 0.1 luminal sodium in 2 c.c. of the solvent liquid. Lippmann treated cases of tabes with lancinating pain and gastric crises, and cases of multiple sclerosis with spasms in the lower extremities, by means of intraspinal injections of 10 c.c. of a 1 to 1.6 per cent. solution of sodium bromide up to three injections at intervals of a fortnight. Other writers in Berlin have been able to confirm Lippmann's favourable reports. I have employed the method in a number of cases and observed that in a few cases of lancinating pain in tabes, and of pains in the legs in Parkinson's disease and multiple sclerosis, an improvement lasting from one to several weeks also set

in when the mere withdrawal of fluid—which sometimes in such cases is alone sufficient—had failed. I was not able to observe any noteworthy effect on spasms. Not infrequently the treatment sometimes provokes violent symptoms of meningeal irritation, but has never produced harm. In encephalitis lethargica the success attending the intraspinal injection of 20 c.c. of polyvalent grippe serum has been occasionally well spoken of (Fendel).

Among medicaments, magnesium sulphate has been injected into the subarachnoid space to the amount of 5 to 10 c.c. of the 15 to 20 per cent. solution on account of its antispasmodic effect in cases of tetanus and eclampsia.

In cases of alarming hæmorrhage in the central nervous system, gelatine (10 c.c. of the 10 per cent. Merck preparation) or adrenalin (10 c.c. physiological saline solution with $\frac{1}{15}$ mg. adrenalin, Kåding) have been recommended. In acute anterior poliomyelitis (Heine-Medin disease) Neisser employs intraspinal injections of strychnine nitrate even for the smallest children, for the prevention of severe paralytic seizures; and Langbein has recommended stovain as a remedy for the pains of sciatica.

The treatment of infectious, chiefly otogenous, meningitis requires special mention. The large number of different methods that have been recommended proves how little satisfaction therapy has hitherto given in this respect. Most writers admit that a certain amount of improvement in the subjective and objective symptoms is to be achieved by frequent lumbar puncture—the withdrawal of larger amounts once or twice a day—and I would not recommend too much reduction of pressure. A few cases of recovery have also been put on record, though, as in all other methods, a *propter hoc* can scarcely be assumed with certainty. The combination with Bier's method of induced hyperæmia of the head has not been extensively adopted, and lumbar drainage

just as little. Bingel extols the aid effected by the insufflation of air (see Appendix).

Irrigation with physiological saline solution (or better with Ringer's solution, as the former has given rise to unpleasant occurrences) whether cranio-caudalward, or, with free communication, caudo-cranialward, would appear to be fairly abandoned; and repeatedly allowing smaller amounts of Ringer's solution to enter and leave seems to be more practised. Serum injections seem to be valueless in otogenous and rhinogenous meningitis, although favourable results have been reported in a few cases. Antiseptics of the most varied sort have been administered intraspinally, evidently without any effect. More recently interest has been awakened for the quinine derivatives (optochin, eucupin, vuzin), whose bacteriological effect was pointed out, as is known, by Morganroth. Linck, Zimmermann, and Huenges have reported on cures from the use of vuzin, which is the most effective of the agents in this series, and is also recommended as a prophylactic. (The technique of the treatment is described in detail by Zimmermann.) Birkholz has brought the cases in the literature of the subject under scrutiny, and has shown, moreover, in cases of his own, that twenty-four hours after the injection of vuzin this agent was not found either at the site of the injection nor in the fluid which flowed from the labyrinth stump left after labyrinth resection. With regard to the wandering upwards of intraspinally injected medicaments the records in the literature are considerably at variance. Eskuchen has shown recently by puncture of the cisterna that serum, i.e., a liquid of higher specific gravity than the spinal fluid, traverses the subarachnoid space from the lumbar cord to the foramen magnum within twenty-four minutes. This does not mean, however, that in meningitis the generally accepted view does not hold good, viz., that the medicament is only distributed over a more or less limited section of the vertebral canal; this view is

supported by Wolf's observations with injections of protargol which provoke pain up to a definite height of the cord. Bingel has attempted to bring the medicament to the brain by withdrawing large amounts of spinal fluid and substituting air (cf. Appendix), after which he injected the medicament in the raised pelvis position, by which the injected fluid flowed to the deeper parts, i.e., towards the head, while the air accordingly rose and escaped by the cannula. Possibly the administration of medicaments by means of puncture of the cisterna according to Eskuchen's simplified technique (see Appendix) will be able to advance our therapeutic endeavours.

The subarachnoid injection of antisyphilitic medicaments has been attempted in many forms for a number of years past, e.g., with various mercurial salts, with colloidal mercury (electromercurol), with mercurialized serum, &c. Salvarsan was first employed by Wechselmann in 1912 for cerebral syphilis, but in his cases spinal irritation was induced. Salvarsan was first employed on a large scale for intraspinal treatment by Swift and Ellis, who reported in 1913 their experience with salvarsanized serum. An hour after the intravenous injection of salvarsan they injected a certain amount of the patient's own serum, mixed with physiological salt solution, into the subarachnoid space. Ogilvie employed serum salvarsanized *in vitro*. The Breslau neurologist Förster, who eulogizes the successes of intraspinal treatment in nerve syphilis, adopts the following routine (cited after Nonne, "Syphilis und Nervensystem," 4. Auflage, 1921):—

"(1) Intravenous injection of neosalvarsan 0.15 to 0.6.

"(2) Half an hour later sterile withdrawal of 100 c.c. blood.

"(3) Standing until the blood-clot has formed, afterwards placing in the refrigerator for eighteen to twenty hours, when the serum becomes fully pressed out.

"(4) Centrifugalization of the serum.

"(5) Inactivation of the serum in the incubator for half hour at 56° C.

"(6) Lumbar puncture: withdrawal of 10 to 20 c.c. of spinal fluid, injection of 10 to 20 c.c. serum by means of a suitable 'Record' syringe.

"The patient remains one to two days in bed. Some patients, however, tolerate ambulatory treatment very well. I repeat the injections every fortnight. During the intervals of salvarsan treatment I inject once or twice a week 1 c.c. of Hirsch's solution, i.e., a 1 per cent. solution of oxycyanide of mercury with an addition of acoin."

Against the employment of salvarsanized serum it is objected that the method is complicated and asepsis is difficult to attain. The attention of many workers therefore has been directed towards administering the salvarsan directly into the spinal fluid of the patient, and Gennerich has carefully elaborated a method with this object in view and has tried it in a large number of cases.

Intraspinal treatment with salvarsan demands the strictest sterility as fatal meningitic infections have repeatedly occurred during its employment. All instruments must be boiled for five minutes in distilled water, and attention must be paid that the tubes are filled with water. Afterwards all vessels and tubes must be rinsed again with freshly distilled water at a boiling temperature.

Gennerich's method is as follows: After withdrawal of the spinal fluid necessary for investigation purposes, the cannula is connected by means of a rubber tubing 40 cm. long with a glass burette or a graduated flask of about 150 c.c. volume. The glass vessel is provided with a stopper. Then a certain amount of spinal fluid, about 30 to 60 c.c., is allowed to flow as slowly as possible into the burette and the stopcock of the cannula closed; should no stopcock be present the tube is closed with a pinchcock. To the spinal fluid contained in the burette

is now added the already prepared solution of neo-salvarsan or salvarsan-soda in the physiological saline solution, or in spinal fluid previously withdrawn for this purpose, by means of a 1 c.c. pipette graduated in tenths and twentieths, and the vessel shaken without allowing the spinal fluid to touch the stopper. Now as much spinal fluid as possible is again allowed to flow as slowly into the glass vessel [burette], in the cases of women up to a total of 40 to 65 c.c. according to their stature ("Grösse"), and for men from 60 to 90 c.c. In paresis it is usually possible to remove as much as 120 to 150 c.c., but in such cases Gennerich advises not to let the whole of the spinal fluid return, but to pour away the excess beyond 70 to 90 c.c. If in the lateral posture the outflow is insufficient in amount, then the patient is allowed to sit up; should violent headache begin, the patient is assisted by a nurse to lie down again, and after recovering a little, again to sit up.¹ From a certain stage onwards the patient will complain of headache—if he does not complain he should be asked—which increases in violence with the further withdrawal of fluid. If the headache can be no longer borne, which in some neurasthenics can be the case after the removal of comparatively small amounts, the withdrawal of fluid must be terminated. If much fluid is removed the pulse must be kept under observation. If a distinct decrease in the pulse-rate occurs spinal fluid must be allowed to flow slowly back again as soon as possible by raising the vessel. Before allowing the fluid to flow back, any air-bubbles should be removed from the rubber tube by

¹ Benedek (*M. med. Woch.*, 1922, No. 2) and Wittgenstein recommend for the readier withdrawal of larger amounts of fluid to perform the puncture in the sitting posture; the fluid is allowed to flow back again in the lateral position. Benedek suggests connecting the cannula with two intercommunicating burettes by means of a glass triangle, in order to secure an intimate mixture of the medicament and to drive out the air; but this may also be achieved by the ordinary method.

stroking it out in the direction of the vessel. Digitalis ("Digalen") must be at hand for emergency in alarming symptoms. If convulsions set in, the trismus must be overcome as soon as cyanosis is present, or artificial respiration must be resorted to if necessary. If perspiration occurs (vagus stimulation) the removal of fluid must be interrupted for one or two minutes. The total length of time taken by the operation is from twenty-five to forty-five minutes.

Recently, Gennerich recommends treatment by double puncture, by which irritation of the cord is obviated, and owing to the lower irritability of the brain much larger doses may be infundibulated—with gradual increase up to 4·5 to 5 mg. The punctures are performed in two places that are separated by one or two intervertebral spaces, and both cannulæ are connected with vessels of which only the upper one receives an addition of salvarsan, while the lower receives pure spinal fluid, and serves the purpose at the conclusion of the operation of washing away towards the head the previously reinfundibulated contents of the upper vessel. The method comes into consideration in all cerebral lesions, but specially for atrophy of the optic nerve. If the cord is to be treated as well the lower vessel must also be given a small addition of salvarsan, about $\frac{1}{3}$ to $\frac{1}{2}$ mg. to 70 to 80 c.c. of spinal fluid. In the double puncture method of treatment first 20 c.c. of fluid are allowed to flow into the upper vessel, which is then shut off; then as much fluid is allowed to flow into the lower vessel as can be obtained, the proper quantity of salvarsan then added to the upper vessel, the contents of the upper vessel allowed to flow back, then about 30 to 50 c.c. of the contents of the lower vessel, and the remaining fluid in the lower vessel is thrown away.

The preparation is dissolved either in 0·4 per cent. saline solution or in spinal fluid, preferably in a sterile watch-glass. The dose of 0·045 grm. of the prepara-

tion is dissolved in 10 c.c. of salt solution, so that $\frac{1}{10}$ c.c. contains 0.45 mg. of the preparation. (If only the dose 0.15 is at hand, then this is dissolved in 15 c.c. of the saline solution, and to 1 c.c. of this dilution—it contains 0.01 gramme of the preparation—is added 9 c.c. of the salt solution, so that 1 c.c. of the second dilution contains 1 mg. and $\frac{1}{10}$ c.c. contains $\frac{1}{10}$ mg. of the preparation. Schubert employs for the dilution of 0.045 of the preparation 3 c.c. of spinal fluid, so that $\frac{1}{10}$ c.c. of the dilution contains 1.5 mg. of the preparation.

A course of treatment consists of a larger series of injections—up to 10 or 12, or more if possible, with two or three injections after the findings have been rendered negative—administered at intervals of two and a half to three weeks, in tabes perhaps four weeks. During the course the dosage is made high at first, with certain exceptions, and decreased after a few injections. (The treatment is combined in addition with an intravenous salvarsan treatment.) The dosage in the single injections, besides depending on the sort of case, depends on the amount of fluid which it is possible to withdraw. The treatment adopted must be individualized to the utmost. The following directions given by Gennerich give the general outline of the course to be followed, from which manifold deviations must be made in each case. The doses to be given in the different groups of disease are : For latent syphilis without nerve symptoms and neurorelapse 1.0 to 1.8 mg., and with double puncture 1 to 5 mg., in fresh cases more than in older cases. Only in cases of epileptiform neurorelapse and in apoplectiform attacks does Gennerich begin at once with large doses (1.8 mg. to 70-90 c.c. of spinal fluid). Cerebral syphilis and paresis at first 1.0 mg., and if no symptoms of irritation arise up to 1.35 mg., and with larger amounts of spinal fluid (above 80-100 c.c.) 1.5, 1.8, 2 mg. In the case of double puncture 1 to 1.2 mg. to begin with, and in

the absence of symptoms of irritation, up to 3 to 5 mg. In *tabes dorsalis* an average of $\frac{1}{2}$ to $\frac{2}{3}$ mg., in disturbances of the bladder and rectum not more than $\frac{1}{2}$ mg., even with amounts of spinal fluid of 60 to 70 c.c., and with amounts of 100 c.c. up to $\frac{3}{4}$ to 1 mg., in ataxia or gastric crises never more than $\frac{1}{3}$ to $\frac{1}{2}$ mg.; double puncture in *tabes* or with severe spinal fluid abnormalities, in non-atactic cases gradually up to 1.0 to 2.5 mg., in atactic and gastric cases gradual increase up to not higher than 1.5 to 2.25 mg.

Gennerich recommends the following chart to be kept always before one during the treatment:—

0.05 c.c. = 0.22 mg. S—Na	} Usual doses in <i>tabes</i>
0.1 " = 0.45 " "	
0.15 " = 0.67 " "	
0.2 " = 0.9 " "	
0.25 " = 1.22 " "	
0.3 " = 1.35 " "	} Usual doses in cerebral syphilis
0.35 " = 1.57 " "	
0.4 " = 1.8 " "	
0.45 " = 2.02 " "	
0.5 " = 2.25 " "	

Bowels and bladder should be evacuated previous to treatment in order that after the operation abdominal straining should as long as possible not be brought into action. Patients who are subject to violent pains in the legs after the removal of larger quantities of fluid should be given an injection of morphia and scopolamine beforehand. After the injection the patient should rest in bed for two days—with double puncture treatment three or four days—the head should be kept horizontal, the feet end of the bed raised, and liquid nourishment given. Rises of temperature, sometimes as high as 40° C. (104° F.) occur, but usually only after the first injection, and they seldom last longer than a day. Sleep is generally disturbed during the first night after the injection. Occasionally after the first injection considerable inflammatory reactions have been observed in the spinal

fluid. Violent symptoms of meningism are not infrequent and occasionally conditions of stupor and convulsive attacks supervene. (According to Gennerich, the latter can be relieved by an immediate intravenous injection of salvarsan, and in certain cases a prophylactic intravenous dose of salvarsan is also commendable.) The symptoms of spinal irritation which sometimes set in are particularly unpleasant—according to Gennerich they result from overdosage—e.g., weakness of the detrusor vesicæ, the sphincter vesicæ and ani, paræsthesia, numbness in the lower extremities, violent pains in the legs, increase of spasms and tabetic ataxia, vesical and gastric crises, and so on; in a few cases they have led to permanent injury.

The successful results achieved by intraspinal salvarsan treatment, which is advocated and has been employed by Gennerich, in a large number of cases, have not yet been finally established but still require detailed clinical verification.¹ At present the method can by no means be recommended for extensive employment in practice. Gennerich considers that its chief field lies in the treatment of inflammatory conditions of the spinal fluid in non-nervous syphilis. If the method is really able

¹ Undoubtedly encouraging results have been obtained by Anna-liese Wittgenstein at the Goldscheider Clinic in Berlin in over 300 cases of treatment by intraspinal infusions. Serious or prolonged injury was not once observed. Healthy conditions were restored in the spinal fluid in every case of non-nervous syphilis treated, even when intravenous treatment had been unsuccessful. Also in a few cases of nervous syphilis and tabes more was achieved than with intravenous treatment alone, hence for many cases Wittgenstein recommends a combination of both methods of treatment. Failure was only observed in paresis. Wittgenstein thinks that intraspinal treatment is entitled to a place among the methods of treating nervous syphilis, but that it does not come into consideration for general use owing to the special training it necessitates and the considerable risk attaching to it.

Schacherl, who stresses that intraspinal salvarsan treatment should only be employed in cases where other therapeutic means have failed, regards this condition satisfied in tabetic atrophy of the optic nerve, the prognosis of which is usually unfavourable, and he reports on a series of cases with comparatively good results.

regularly to overcome spinal fluid abnormalities, which general treatment is so often powerless to influence, then it would indeed prove to be highly important. For this reason, I have considered it not out of place to give a more detailed description of the method. For it is syphilis which supplies us with the most important problems for the subject of the present book.

APPENDIX.

A.—ON ENCEPHALOGRAPHY.

IN 1918, Dandy's first publication appeared on the insufflation with air of the ventricles: Ventriculography. A short time afterwards followed the attempt by the same writer to get an X-ray picture of the cerebro-spinal fluid spaces by injecting air into the vertebral canal; but Dandy soon returned to his original method. Independently of each other Bingel and Widerøe reported in 1920 on the results obtained from the intraspinal insufflation of air, to which Bingel gives the name encephalography. A number of publications on these methods have appeared in recent years; they formed the subject of a paper by Weigeldt, and a lively discussion at the Annual Meeting of the Society of German Neurologists in October, 1922.

The technique of spinal insufflation, according to Bingel's original method, which is also recommended by Weigeldt, is exceedingly simple. It is performed by alternately withdrawing 5 to 10 c.c. of spinal fluid, continuously controlling the pressure, and introducing the same volume of unfiltered air by means of a "Record" syringe, which is attached airtight to the cannula. All that is required, therefore, besides the ordinary instruments necessary for lumbar puncture is a syringe holding 10 c.c., and a measuring glass in which to collect the fluid. A more complicated method of double puncture described by Bingel, in which the air pressed from a bottle through one cannula produces a flow of an equal volume of spinal fluid from the other cannula, is no doubt unnecessary. The employment of carbonic

acid gas seems to have been abandoned by Bingel himself. Nor would the use of oxygen, for which Schinz has described an apparatus (*Zentralbl. f. Chir.*, 49, 1922, 1367) appear to offer any special advantage.

Owing to the danger of vomiting the puncture is performed in a fasting condition, and to aid the ascent of gas, in a sitting posture of the patient. Bingel advises the administration of $\frac{1}{6}$ to $\frac{1}{3}$ gr. of morphia and $\frac{1}{3}$ to $\frac{2}{3}$ gr. of scopolamine one hour beforehand; Weigeldt warns against this owing to the danger of collapse. The operation is perceived by the patient, excepting quite indolent patients (paresis, apoplexy), as a peculiar feeling of gargling, buzzing or bubbling; perspiration regularly breaks out; also more or less violent headache, sometimes very violent, and not infrequently nausea, dizziness, vomiting occur, sometimes accompanied by increased or decreased pulse-rate and collapse (horizontal position and cardiac stimulants!); Bingel once saw an epileptiform attack. The amount of air introduced is usually 100 c.c. after the withdrawal of an equal volume of spinal fluid. The symptoms of meningeal irritation which usually follow, also occasionally rises of temperature, generally disappear in two or three days, or sometimes sooner.

The method is not free from danger, and a few fatal cases have occurred in tumours of the brain (posterior cranial fossa), evidently of the same origin as the similar mishaps in simple lumbar puncture. As Bingel saw a case with such a termination—though every *post hoc* here is not a *propter hoc*—in which the pressure remained constant during the whole procedure, the operation must be regarded as absolutely contra-indicated in cases of suspected tumour in the posterior fossa. In such cases only insufflation of air by proceeding from the ventricles comes into consideration; and the same applies to cases in which the communication between the ventricles of the brain and the subarachnoid space is closed. Insufflation from the ventricles is in general preferred by

some writers, but it has undoubtedly other disadvantages owing to the greater difficulty and danger attaching to the operation itself. As a few fatal cases have occurred in encephalography in other groups of diseases (a case recorded by Goldstein, an advanced case of paresis by Trömmner), the condition is well justified that the method is only applicable within the strictest indications, and only when the clinical investigation has broken down; with patients in a weak condition the method is to be dissuaded from.

The interpretation of the X-ray findings is often difficult, and, as Schüller points out, there are also other sources of error which arise from the imperfect insufflation of air owing to fluid remaining behind in the cerebro-spinal fluid spaces, and from mistaking lighter parts, due to cranial sinuses, thinning of the cranial wall and cancellous spaces of the base of the skull, for air-filled spaces. The typical picture of a ventricular insufflation with a transverse X-ray exposure shows the butterfly-shaped figure of the lateral ventricles in the middle of the field, between them the distinct line of the septum pellucidum curving away from the columnæ fornicis, and directly below it the oval, axially-placed field of the third ventricle. The subarachnoid space of the brain is also represented. Lateral exposures also, of course, provide serviceable skiagrams. In the region of the cord Bingel succeeded in rendering the cisterna terminalis and the cervical part visible, but not the intermediate parts, owing to the thinness of the cylinder of air and the absorption of the X-rays by the trunk mass.

The chief uses of the method are in lateral diagnosis [localization] of tumours of the brain and in diagnosis of the height of tumours of the cord. The chief symptoms in tumour of the brain are in the diminished insufflation of the subarachnoid space of the same side, the displacement of the intercerebral fissure or the septum pellucidum to the opposite side; the ventricle of the affected side is

usually deformed and reduced in size, that of the other side generally enlarged. Sometimes local changes of form of the ventricles are able to supply closer details for the local diagnosis. In the case of tumours of the cord, the occurrence of pain during the insufflation—purposely made to vary in the degree of pressure—is a reliable symptom for the localization of the lesion; this pain, however, is also found to occur in cases of intramedullary tumour (Bingel), as well as in shallow angioma and meningitis serosa (Marburg). For the rest, the determination of the amount of fluid situated below a totally occlusive lesion provides an indication of its situation (cf. amount of spinal fluid, p. 4 *note*). Josefson succeeded in locating a total occlusion by means of X-rays.

Therapeutical successes attending the insufflation of air are also extolled, e.g., in epilepsy (Bingel, Weigeldt), in severe puerperal eclampsia (Bingel). Bingel and Curschmann call attention to the marked improvement obtained in the subjective and objective symptoms in cerebral tumour.

B.—PUNCTURE OF THE CISTERNA CEREBELLO-MEDULLARIS.

Methods of gaining access to the cisterna magna for therapeutic and diagnostic purposes by the performance of smaller or larger operations have been described by a number of investigators. Eskuchen has recently followed with a simple technique for puncturing the cisterna which may ultimately become a valuable addition to lumbar puncture, partly in rendering assistance in particular cases to local diagnosis, and on the other hand as a means of medicinal treatment in infectious meningitis, perhaps also in the salvarsan treatment of nerve syphilis. Eskuchen found injections of salvarsanized serum to be innocuous.

Eskuchen describes the technique as follows :—

“The patient sits with the body bent slightly forwards ; the head is held by an assistant, without, however, being inclined forward. In order to obtain a better view and for disinfection the depression below the occipital protuberance is shaved in the median line about 2 in. long and $\frac{1}{2}$ in. broad. From the protuberance the bone is palpated downwards to the spot where the finger is pushed aside from the bone by the lig. nuchæ, mark I. Then the position of the tuberc. post. atlant. is sought to be determined by palpation, mark II. This succeeds without difficulty in lean persons, while with stouter persons one must be satisfied with finding the epistropheus process. Midway between mark I and mark II, which is usually about 1 cm. below mark I, the puncture is performed by introducing the needle *in the direction towards the supposed end of the internal occipital protuberance* (*‘vermutliche Ende der Hinterhauptschuppe’*). An ordinary, well-pointed cannula, as used for lumbar puncture, is employed, but of a smaller calibre, and one whose stylet can be quite removed and a “Record” syringe adjusted. If the pressure conditions are to be investigated a cannula provided with a side branch and an air-tight stylet are necessary. The perforation of the lig. nuchæ necessitates a certain pressure, in exercising which counter-resistance is applied at the same time (*‘wobei man gleichzeitig gegenhält’*) in order not to penetrate too deeply unawares [i.e., the cannula must be kept under perfect control]. The most important part of the whole puncture is to succeed in reaching the posterior margin of the foramen magnum. It is better to strike the bone too soon than to miss it altogether. When the bone is felt at the point of the cannula, one must feel the way, by slightly raising the cannula-handle, along the bone to the foramen. The point at which the internal occipital protuberance ends at the foramen is the *point of orientation* for the puncture. *It must be*

touched under all circumstances. Directly on the margin of the foramen magnum the cannula is now advanced in the direction gained and the membr. atlanto-occipitalis perforated. From the bony directing point one has still to advance as a rule about $\frac{1}{2}$ to $1\frac{1}{2}$ cm. (a half to one-and-a-half centimetres). One usually feels the membrane being pierced, and above all, the entry into the cistern, from the distinct cessation of all resistance (as in lumbar puncture). As there is no absolute reliance on the orientation from the sense of touch, owing to the often powerful resistance offered by the lig. nuchæ to the advance of the cannula, it is best to aspirate tentatively, after having removed the stylet, at every few millimetres' advance of the cannula. On no account must the cannula be advanced forward considerably without this test ('ohne Kontrolle'). After having entered the cisterna a few drops of fluid are aspirated for the cell-count, and then slowly the amount needed for the other investigations is aspirated (10 to 20 c.c.); finally a few more drops for a second cell-count. If the patient's head is caused to be inclined forwards a certain amount of fluid may be easily aspirated; this is almost sure to be sub-arachnoid fluid and is from the immediate neighbourhood. If, however, it is desired to remove a larger quantity of fluid (e.g., for irrigation purposes), it is better to cause the head to be raised; the fluid thus obtained is at least partly, if not entirely, ventricle fluid that flows, or is aspirated, from the for. Magendie.

"As to the distance to which the needle must be advanced, no definite details can be given. According to condition of nutrition, age and sex of the patient, the cisterna is to be reached sometimes at a distance of 4 cm., sometimes not less than 7 or 8 cm. I have most frequently measured an average of 4.5 to 5.5 cm. The orientation is somewhat more difficult in the lateral posture, but it has the advantage that the fluid need not be aspirated. If a very narrow cannula is employed,

the stylet can be definitely removed after having passed the edge of the foramen, and the cannula advanced further with attached syringe (in the sitting posture) or without syringe (in the lateral position). Whether to decide to perform the puncture in the lying or sitting position depends entirely on the purpose of the investigation. If, e.g., the purpose is to establish or to exclude the presence of an obstruction of the spinal subarachnoid space, then puncture in the lateral position is by far the best. If, on the other hand, information is desired on the condition of the 'local' fluid, then puncture in the sitting position is to be preferred. Patients, too, usually prefer the latter position. Local anaesthesia is unnecessary, and not even advisable, owing to the orientation being rendered more difficult. Sensitive patients are given morphia $\frac{1}{6}$ -gr. or dicodid $\frac{1}{6}$ -gr. The treatment subsequent to cistern puncture coincides with that after lumbar puncture."

Eskuchen never saw the slightest untoward symptoms in any single case of cisterna puncture. He considers it absolutely contra-indicated in cerebral tumour. For the rest he recommends special attention to be paid to the following points:—

"1. In every puncture contact *must* be made with the middle of the posterior margin of the foramen magnum—it constitutes the point of orientation.

"2. The cannula must not be advanced more than at most $1\frac{1}{2}$ cm. beyond this point.

"3. If the orientation point is not found, or if no fluid is obtained at a distance of $1\frac{1}{2}$ cm. past this point, the puncture must be discontinued. For if one advances deeply without being oriented by the bone, one is absolutely in the dark as to the position of the cannula. If, on the other hand, no fluid is obtained at $1\frac{1}{2}$ cm. after having passed the margin of the foramen, then some special conditions must be present in the cisterna which only render the withdrawal of fluid possible, perhaps, in

dangerous proximity to the medulla. If, therefore, the bone is not arrived at at the expected depth (a graduated cannula should be used), the cannula must be given a direction inclining somewhat more upwards after having withdrawn it a little. But if the bone has been met at too obtuse an angle so that it cannot be passed alongside the bone without difficulty, then the direction of the needle must be changed in the opposite manner. The cisterna magna has a depth of at least $1\frac{1}{2}$ cm.; if the directions are adhered to, all possibility of injuring the medulla is eliminated. Injury to the cerebellum is likewise impossible unless this has been displaced by a space-diminishing lesion. The danger of lacerating the membr. atlanto-occip. is also absent provided a sharp cannula is employed."

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